THE ENDOCRINE CONTROL OF EGG PRODUCTION IN POULTRY

by

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DECLARATION

I declare that this thesis has been composed by myself and that it consists of the results of my own work.

CONTENTS

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SUMMARY

1.

(i) Yellow yolky ovarian follicles were shown to be an important, though not the sole source of progesterone in laying birds. Levels of progesterone and LH in the plasma were directly related to the extent of ovarian follicular development. However, during the brooding period an increase in plasma LH levels is not associated with an immediate increase in plasma progesterone levels.

(ii) The largest, mature, yellow yolky follicle seems to produce more progesterone than smaller follicles and progesterone from this source may be largely responsible for the pre-ovulatory increase in plasma progesterone levels.

(iii) A combination of a diurnal increase in plasma LH levels and other changes in plasma concentrations of other hormones (possibly androgens or prolactin) was suggested to initiate progesterone secretion by the mature, pre-ovulatory ovarian follicle. Increased progesterone secretion triggers the pre-ovulatory increase of LH acting via a positive feedback system which has an important neural component.

(iv) Hens were observed to lay shorter sequences of eggs as they become older, but as the times of lay of first and last eggs of a sequence did not vary with age, shorter sequences were not due to a shortened 'open period' of the ovulatory cycle. The higher rate of lay of modern egg-type strains compared to strains used over twenty years ago cannot be explained by differences in the times of lay or lag within a sequence. Times of lay and lag were identical in both types of hen.

(v) An age-dependent shortening of sequence length was observed in three different strains of hen (Ross I broiler breeder; Ross Ranger mid-weight egg layer; Babcock B300 lightweight egg layer) with markedly different egg production rates. It

was suggested that this may result from an increase in the time taken for successive follicles to reach an ovulable condition. The ovulable condition, or the maturity of the follicle was thought to be related to its capacity to secrete, and sustain secretion of large amounts of progesterone sufficient to activate the positive feedback mechanism controlling LH release. This maturity was independent of the size of the follicle and the amount of yolk it contained.

(vi) A further cause of declining egg production with age in all three strains studied was the occurrence of short breaks in laying of 2-7 days. These breaks may have been due to undetected soft-shelled eggs, internal ovulation or occasional follicular atresia. Longer breaks in laying, of the order of 28 days, were an important source of lost production in the broiler breeder and mid-weight egg laying strains. The relatively early incidence of long breaks in broiler breeders may account for the poor overall egg production of these hens.

(vii) The pattern of LH levels in growing hens was different in all three strains. All strains showed a pre-pubertal peak of LH associated with the development of the progesterone positive feedback mechanism, but this occurred at a different time in each strain relative to the onset of lay. The mean plasma LH levels were higher in developing Ranger than Ross I or Babcock hens. Rising plasma progesterone titres were associated with the onset of lay, but they were not associated with the decline in plasma LH titres from peak pre-pubertal values. It was suggested that the pattern of pre-pubertal LH secretion, by affecting early ovarian follicular development, may have a bearing on subsequent egg laying performance.

(viii) The basal progesterone and LH plasma levels during the first laying year differed in the three strains. Furthermore, the fluctuating basal hormone levels did not correlate with each other, nor could basal hormone levels be correlated with egg production in any way. It was suggested that basal levels of

gonadotrophins may regulate the incidence of follicular atresia.

(ix) The **morphology** of the ovaries of broiler breeder and egg laying type hens are similar and age-related changes in the ovarian follicular hierarchy are seen in both strains. Follicles grow to a larger size in old birds before they ovulate, but this may only have a minor effect on egg production rates. Abnormal, irregular laying patterns seen in old hens were not the result of abnormal, irregular follicular hierarchies.

(x) A further cause of the decline in egg laying in older hens appeared to be a reduction in the sensitivity of the positive feedback mechanism governing LH release. The pituitary response to LH-RH and the ovarian progesterone secretion response to LH were unchanged in older hens. It was suggested that the increase in the time taken for follicles to mature in older hens allows a greater capacity for progesterone secretion to develop so that the less-sensitive positive feedback system can nevertheless be activated to trigger pre-ovulatory LH release.

2. INTRODUCTION

2. INTRODUCTION

The egg-laying capacity of the domestic hen has improved dramatically over the past four or five decades. Doubt has been expressed, however, as to whether this improvement is due to the efforts of geneticists in selecting for egg production, or to the elimination of inferior stocks and technological improvements, such as the provision of artificial lighting (Clayton, 1972). Geneticists have been notably successful in the selection of fast-growing birds for meat production (Clayton, 1972). However, selection for weight and broad-breasted conformation is associated with declining reproductive performance (Merritt, 1968). Figures obtained from the Ministry of Agriculture's Agricultural Development and Advisory Service in 1977 illustrate the egg-laying capacity of egg-type and meat-type birds, kept under commercial conditions. A good egg-laying strain, the Babcock B300, reaches peak rates of 84.7% but declines to 71.3% towards the end of the laying year. The comparable figures for the Ross I broiler parent stock are 78.6% and 52.6% respectively. Interestingly, no hen can match the laying performance of the Khaki Campbell duck which, according to Hutt (1952), will average greater than 85% egg production all through the first laying year. Furthermore, the Khaki Campbell duck weighs about the same as the domestic hen yet lays eggs which are 20% larger. It is possible, therefore, that even high-producing hens are capable of still greater egg productivity.

A greater understanding of the anatomical and physiological changes in the female's reproductive system associated with ageing may help define an approach to the problem of declining egg production as birds grow older. In this thesis, advantage has been taken of modern techniques making it possible to measure levels of reproductive hormones

in the blood of the hen, and of recent advances in knowledge of avian reproductive physiology to investigate this problem.

2.1 Morphology of the Ovary in Laying Hens

The avian ovary consists of many oocytes contained within the stroma, and organised into follicles. Each oocyte is surrounded by a layer of granulosa cells which in turn is surrounded by layers of connective tissue termed the theca. There is a continuous development of follicles with one follicle reaching maturity approximately every 24 to 27 hours. In the final 9 to 11 days of development, follicles accumulate yolk at an increased rate and grow rapidly (Gilbert, 1972). This rapid growth phase gives rise to a hierarchy of follicles which can be described by size or weight. The hierarchical arrangement of the five or six largest follicles, containing yellow yolk, is easy to observe but it is not so easy to see how the smaller follicles are arranged. However, Gilbert (1971) has shown that the hierarchy can be traced back in the follicular maturation process to small, yellow-white yolky follicles. These follicles are selected from the pool of slowly-developing follicles in an ordered fashion. How follicles destined to ovulate are selected from the pool of undifferentiated follicles is uncertain, but the maintenance of the hierarchy of growing follicles is a finely controlled, easily disrupted process which appears to depend on levels of gonadotrophic hormones in the blood. For example, the regressed ovaries of hypophysectomized hens, or the regressed ovaries of intact hens pre-treated with an anti-gonadotrophic drug, can be stimulated to a near normal development of the hierarchy by daily treatment with 50 mg avian pituitary powder (Mitchell, 1967a, 1967b). The dose of hormone required is critical since 20 mg/day of avian pituitary powder consistently resulted in

a smaller than normal hierarchy while overdeveloped ovarian hierarchies were observed in some hens receiving 50 mg/day. Supradevelopment of the normal follicular hierarchy can be induced by the injection of gonadotrophin in intact hens (Nalbandov, 1959). In view of these data it would be helpful to ascertain if gonadotrophin blood levels and/or the structure of the follicular hierarchy differ between old hens laying few eggs, and young hens laying many eggs. One may also ask if the relatively poor egg-laying performance of broiler breeders when compared with commercial egg-laying strains is attributable to differences in levels of circulating gonadotrophins, or to differences in ovarian structure. An intriguing observation by Jaap & Clancy (1968) that the ovaries of broiler-type hens contain more yellow, yolky follicles than those of a commercial egg-type strain suggests that the relatively poor egg-laying performance of broilers is not due to a lack of mature, ovulable follicles.

2.2 Reproductive Hormones in the Hen

Only the left ovary becomes functional in the hen and its growth, differentiation and activity are controlled by the adenohypophyseal gonadotrophins. This was first shown by Nalbandov & Card (1943). Hypophysectomy of adult hens led to atrophy of the gonads. It was found that this effect could be reversed by the daily injection of chicken anterior pituitary powder (Nalbandov *et al.*, 1951; Opel & Nalbandov, 1961). Gonadotrophic substances were identified in the anterior pituitary of 18-week-old chicks by Moszkowski (1949), and it is now known that the pituitary gland produces two gonadotrophins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). This

has been established by the partial purification of anterior pituitary gland extracts and the bioassay of the resulting fractions (Stockell-Hartree & Cunningham, 1969; Furuya & Ishii, 1974). The existence of chicken FSH and LH as separate moieties with different biological actions has also been confirmed by injecting hypophysectomized male birds daily for 2 weeks with either an FSH or an LH chicken pituitary fraction, and examining the degree and type of gonadal development by light and electron microscopy (Brown et al., 1975). In the female, it is thought that FSH regulates follicular development (Mitchell, 1967a,b) while LH regulates steroidogenesis (Shahabi et al., 1975a) and ovulation (Fraps et al., 1947).

Prolactin is another hormone which has been isolated from chicken anterior pituitary tissue, and the resultant preparation has been used to develop a specific radioimmunoassay (Scanes et al., 1976). These authors have shown that plasma prolactin levels tend to be higher in incubating hens. A more recent study has revealed a decline in plasma prolactin levels during the ovulatory cycle of the hen, the nadir occurring about 8h prior to ovulation (Scanes et al., 1977).

The release of LH is controlled by a releasing hormone, luteinizing hormone releasing hormone (LH-RH) produced in the brain and released into the pituitary portal vessels. Hypothalamic extracts from quail have been shown to contain LH-releasing activity (Follett, 1970; Smith & Follett, 1972). Also, immunofluorescent material has been observed in the median eminence of the duck (Calas et al., 1973) and the cockerel (de Reviers & Dubois, 1974) using antisera to synthetic LH-RH. Synthetic LH-RH will cause the release of LH in the chicken (Bonney et al., 1974). Chicken LH-RH must be similar or identical to synthetic LH-RH as hypothalamic extract from cockerels cross reacts in a radioimmunoassay (RIA) for LH-RH and shows similar

properties to synthetic LH-RH when subjected to thin-layer and ion-exchange chromatography (Jeffcoate et al., 1974).

The ovary of the domestic fowl produces a wide range of steroid hormones (review: Gilbert, 1971). Much current interest has focussed on progesterone, oestrogens and testosterone whose circulating levels have been shown to vary during sexual development and during the ovulatory cycle (progesterone: Furr, 1973; Furr et al., 1973; Haynes et al., 1973; oestradiol 17β and oestrone: Senior, 1974a,b; Senior & Cunningham, 1974; testosterone: Etches & Cunningham, 1977).

2.3 Negative Feedback

Since ovarian activity is regulated by the pituitary which in turn is controlled by the hypothalamus, it is convenient to refer to this system as the hypothalamo-pituitary-ovarian axis. The ovary, while dependant on pituitary gonadotrophin secretion, influences the production of LH through the 'feedback action' of its steroid secretions. A negative feedback effect of sex steroids which restrains LH secretion to basal levels has been demonstrated in the chicken by Wilson & Sharp (1975a). They showed that ovariectomy results in a tenfold elevation of mean plasma LH levels in the hen, and castration results in a fivefold elevation in the cockerel. Further, LH plasma concentrations do not remain steady in gonadectomized birds but exhibit frequent variations which are due to episodic release of LH (Wilson & Sharp, 1975a). Wilson & Sharp (1976b) also showed that administration of either progesterone or oestradiol benzoate, or a combination of the two hormones, to an ovariectomized hen reduces plasma LH levels to around those of an intact laying hen. There are indications that this treatment also abolishes episodic secretion. A

positive feedback effect of progesterone, stimulating LH secretion during the ovulatory cycle, is discussed in a later section (The Hormonal Control of Egg Production: The role of steroids (See Fig 1).

2.4 The Ovulatory Cycle of the Hen

2.4.1 Characteristics of the egg-laying pattern

A sexually mature hen maintained under a 24h light/dark cycle lays eggs at intervals of around 26 hours in a sequence of two or more. The first egg of a sequence is laid soon after the lights come on and subsequent eggs are laid progressively later in the day until the last egg of the sequence is laid in the afternoon. On the following day, no egg is laid and this is termed a pause day. Normally, only one pause day intervenes between successive sequences, and such sequences are said to be 'coupled', but 'uncoupled' sequences with more than one pause day intervening are also observed. Any hypothesis attempting to explain the hormonal control of this cycle must account for the difference in time of day or 'lag' between consecutive oviposition times, and for the phenomenon of pause days which are concomitant with the resetting of the whole cycle.

2.4.2 The influence of light on egg-laying patterns

The pattern of egg-laying described above is synchronized by time-keeping clues in the environment. Commonly, diurnal changes in illumination provide such clues.

Thus, flocks of hens kept in continuous light lay at random throughout the 24h day (Warren & Scott, 1936; McNally, 1947; Morris, 1961) and environmental clues such as regular feeding times or

noise (McNally, 1947; Wilson et al., 1964) cause the birds to synchronize their laying when the lighting is constant. Individual birds kept under constant light lay at high rates not significantly different from those rates of lay observed under 23, 24 and 27 hour cycles (Morris, 1973).

Morris (1973) has drawn a clear distinction between the entraining, or phase-setting properties and the photoperiodic effects of the light cycle. Many physiological phenomena show diurnal rhythms in their manifestation; that is they occur or show increased activity at approximately 24h intervals in a constant environment. When the environment provides constant, time-locked clues (such as sunrise or sunset) such diurnal rhythms become locked to the 24h solar cycle, and the rhythm is then said to be entrained by the environmental clue. Thus, a lights on or lights off signal in an artificially illuminated hen house may entrain the time of egg-laying, while any effect on egg production which can be shown to depend on the duration of the photoperiod would be termed a photoperiodic effect.

In the hen, the important feature of the entraining effect of light appears to be sunset, or under conditions of artificial lighting, the time the lights are extinguished. Thus, when the length of the light/dark cycle is varied using a fixed photoperiod (e.g. 14L:10D to 14L:11D), the egg output is little affected, but the time of oviposition as measured from the onset of the dark period is markedly affected (Biellier & Ostman, 1960). Also the timing of the pre-ovulatory LH surge is advanced with respect to the dark period if hens are maintained on the usual 14h photoperiod incorporated in a 27h light/dark cycle (Morris et al., 1975). On the other hand, if the length of the photoperiod is varied within a fixed cycle, then there is no change in the time of lay counted from the onset of darkness (Lanson, 1960), but the egg production can be profoundly affected

(Morris, 1967).

It must, however, be noted that the photoperiodic effects of light are complex and affect other processes too. The length of the daily photoperiod affects the age of sexual maturity as well as the rate of egg output, and there is evidence (Morris, 1967) to suggest that the experience of a previous photoperiod, or transfer from one photoperiod to another rather than the photoperiod itself are important. Morris (1973) concluded that the photoperiod may affect the supply of ripening follicles in the ovary and perhaps, more speculatively, the 'baseline' rates of FSH and LH secretion; while the phase-setting properties of the light cycle control the timing of the pre-ovulatory release of LH. Further support for this last contention may be obtained from the work of Wilson & Sharp (1973) who commented on the close relationship between the pre-ovulatory LH peak and the dark period in hens kept under a 24h light/dark cycle (14L-10D).

2.4.3 The effects of light on the ovary and ovulation

Although the intervening physiological mechanisms remain obscure, the various observations cited above concerning the entrainable nature of patterns of egg-laying suggest light may affect the rate of follicular maturation and the timing of the pre-ovulatory release of LH, possibly by synchronizing some endogenous biological 'clock'. There must presumably be some communication system linking ovarian follicular maturation and the LH release mechanism as ovulation depends on their synchrony. For example, does the brain send a signal to the ovary to inform it that ovulation should take place, or does the ovary signal its readiness to ovulate to the brain, and might any such signal consist of a surge of hormone secretion? Morris (1973) has demonstrated the independence of the LH release system and the ovarian maturation

timing system. A 27h light/dark cycle will alter the timing and frequency of egg-lay without affecting the rate of yolk output. However, 30h or 21h cycles so limit ovulation frequency that total yolk output is reduced. It appears likely that the rhythm of ovarian follicular maturation has a non-circadian periodicity of 26 or 27h, as hens subjected to such day lengths lay eggs continuously with only occasional pause 'days' (Byerley & Moore, 1941; Biellier & Ostmann, 1960). Also, the mean interval between eggs laid in short sequences under 24h days tends to be around 26h (Atwood, 1929). The inability of hens to lay continuously, and the increase in the interval between successive eggs within sequences under constant lighting conditions (Morris, 1961) lends support to this idea and suggests that the rhythm of follicular maturation has a somewhat longer periodicity than the entrainable rhythm of LH release. Eventually, the phase of ovarian maturation becomes so advanced it cannot interact with the entrainable rhythm of LH release and ovulation cannot occur. This suggests that a comparison of follicular maturation rates in broiler and egg-type hens, also in young and old hens, might be of interest. Any increase in the time taken for successive follicles to come to maturity could account for a decreased egg production rate in ageing hens. While the idea of a biological clock as controller of follicular maturation (van Tienhoven & Planck, 1974) is an attractive one, such ideas are speculative. A key property of an endogenous circadian rhythm, as defined by Bunning (1967) is an approximate 24h periodicity under constant environmental conditions. Elliot *et al*. (1964) have provided clear evidence of this property in the Golden Ham ster. In the chicken, there is no evidence that the entrainable processes of egg-laying and ovulation free-run to an approximate 24h rhythm in a constant environment.

2.4.4 Hormonal Control of Egg Production

2.4.4.1 Role of LH

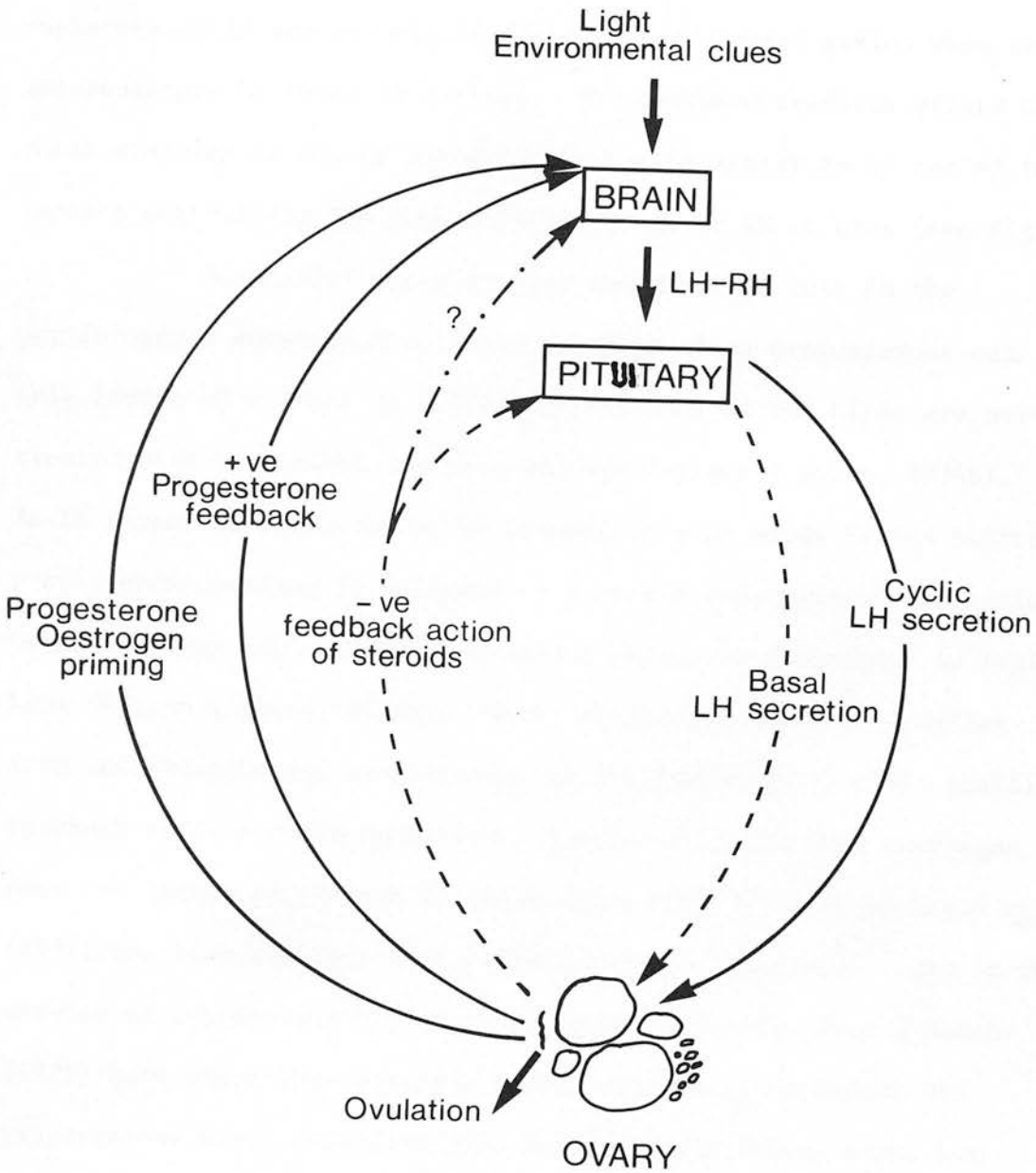
Ovulation can be induced by a judiciously timed injection of a gonadotrophic preparation, such as anterior pituitary powder (Fraps, Riley & Olsen, 1942) or pregnant mare's serum (Fraps, Olsen & Neher, 1942). Generally, the responsiveness of the follicle increases as the interval between the previous ovulation and injection is increased from 7 to 19h while ovulation of the first follicle of a sequence can be experimentally advanced by as much as 20-24h before the expected time of ovulation (Fraps, 1946). This shows that the non-occurrence of ovulation resulting in a pause day is not due to the inability of the follicle to respond to gonadotrophins. Ovulation-inducing hormone is thought to be LH because a partially purified pituitary LH fraction was found to be 500 times more potent than an FSH fraction in inducing ovulation (Fraps et al., 1947). Mammalian and fowl LH have been shown to have similar chromatographic properties and chicken LH is active in the rat ovarian ascorbic acid depletion (OAAD) bioassay (Stockell-Hartree & Cunningham, 1969). It has also been found that a single injection of an antiserum against partially purified LH will block ovulation for 5 days and bring about follicular atresia in laying hens (Sharp, Scanes & Gilbert, 1978). In addition, direct measurement of LH by radioimmunoassay has revealed a peak plasma concentration about 6h before an ovulation (Wilson & Sharp, 1973).

2.4.4.2 The role of steroids

LH is the hormone which directly induces ovulation but certain steroid hormones, through an indirect action, can also force an ovulation. Injections of progesterone (Fraps & Dury, 1943; Wilson & Sharp, 1976c) and testosterone (Fraps, 1955; van Tienhoven, 1961) cause

Fig 1

A diagrammatic representation of the relationships in the hypothalamo-pituitary-ovarian axis in the mature, laying hen.


Light
Environmental clues
BRAIN
LH-RH
?
PITUITARY
+ve
Progesterone
feedback
Progesterone
Oestrogen
priming
– ve
feedback action
of steroids
Cyclic
LH secretion
Basal
LH secretion
Ovulation
OVARY

premature ovulation in the mature laying bird, and either steroid is capable of causing LH release when injected into laying hens (Wilson & Sharp, 1975b, 1976a). These authors have shown that testosterone is only able to stimulate LH release when the ovary contains a mature ovulable follicle. Progesterone is effective at most stages of the ovulatory cycle and is only ineffective for a short period when the pre-ovulatory LH level is falling. The positive feedback effect of these steroids on the LH secretion mechanism appear to be one of the factors controlling the pre-ovulatory surge of LH in hens (see Fig. 1).

Oestradiol may also play an important role in the physiological events controlling ovulation since progesterone can only induce LH release in ovariectomized hens if the birds are pre-treated with oestradiol and progesterone (Wilson & Sharp, 1976b). An LH surge, however, cannot be induced in such birds if the oestrogen/progesterone priming is followed by a single injection of oestradiol benzoate. Nor can oestradiol benzoate induce an LH release in laying hens (Wilson & Sharp, 1976a). Thus, oestradiol appears to differ from testosterone and progesterone in its inability to exert positive feedback effects on LH secretion. Further evidence that oestrogen does not induce LH release directly comes from the work of Laguë et al. (1975) who have reported that ovulation can occasionally occur in the absence of a pre-ovulatory oestrogen peak. Finally, Furr & Smith (1975) have shown that antisera raised against testosterone and progesterone block ovulation when injected into laying hens, but injections of an anti-oestrogen serum have no effect.

2.4.4.3. Control of the pre-ovulatory hormone surges.

From the results of hypophysectomies performed at various

intervals between 2 and 10h prior to the time of an expected ovulation (Rothchild & Fraps, 1949), it was concluded that the release of ovulation-inducing hormone must occur about 4-6h before an ovulation The measurements of plasma LH using Parlow's (1961) OAAD bioassay tended to confirm this (Nelson et al., 1965; Bullock & Nalbandov, 1966; 1967) but at the same time the bioassay revealed two other peaks in plasma at 21h and 13-14h before ovulation. Jackson & Nalbandov (1969) subsequently showed that this bioassay is not specific for LH but probably also measures arginine vasotocin too. Using a homologous RIA developed by Follett, Scanes & Cunningham (1972), only one peak of plasma LH was observed during the ovulatory cycle of the hen between 4 and 7h before ovulation (Furr et al., 1973; Wilson & Sharp, 1973).

Initiation of the pre-ovulatory LH surge

The ability of certain steroids to exert a positive feedback on LH secretion has been discussed above. As the levels of plasma progesterone (Furr, 1973; Haynes et al., 1973; Furr et al., 1973), oestradiol 17β Senior & Cunningham, 1974), oestrone (Senior, 1974b), and testosterone (Schrocksnadel & Bater, 1971; Etches & Cunningham, 1977) all rise to a peak at between 4 and 8 hours before ovulation in approximate synchrony with LH, it is tempting to envisage that the positive feedback effect of steroid hormone is involved in the initiation and maintenance of the pre-ovulatory LH surge. Nonetheless, a single injection of ovine LH can elevate plasma titres of testosterone and progesterone, though not oestrogens (Shahabi, Bahr & Nalbandov, 1975) so it is equally possible that the pre-ovulatory surges of steroids are initiated and maintained by a gonadotrophic stimulus. Simultaneous measurements of LH and steroids have not so far clarified the issue. According to Furr et al., 1973) progesterone concentrations

rose concomitant with, or shortly before the rise in LH when hourly blood samples were taken. Also, Laguë et al. (1975) using 20 min. sampling intervals were unable to determine if the rise in LH preceded that of progesterone. Senior & Cunningham (1974) found that oestradiol levels started to rise about 2h before LH while Laguë et al. (1975) reported that LH and progesterone peaked at the same time as oestradiol and oestrone. In summary, no definite conclusions can be made regarding the role of steroids in the initiation of LH release. It would be useful to measure progesterone, testosterone and LH in the same plasma sample, and take samples frequently during the ovulatory cycle in an attempt to clarify this important issue.

2.4.4.4. Endocrine functions of the pre-ovulatory follicle

There is some evidence that the pre-ovulatory peaks of steroids may be due to synthesis and secretion by major ovarian follicles. Furr (1973) has reported levels of progesterone 8-50 times higher in follicular venous plasma than in peripheral blood collected simultaneously, while Shahabi, Norton & Nalbandov (1975) have shown that peaks of progesterone content of the largest follicle, testosterone content of the three largest follicles, and oestrogen content of the third largest follicle all occur in synchrony with the peak peripheral plasma titres of these hormones 4-7h prior to ovulation. It has also been shown that testosterone and progesterone, but not oestrogen, concentrations in the follicle walls increase following a single intravenous injection of 25μg ovine LH given at a time when no natural peaks of plasma steroids occur (Shahabi, Bahr & Nalbandov, 1975). The failure to stimulate an increase in oestrogen concentration was thought by these authors to indicate that the plasma peaks of

oestrogens seen prior to ovulation are not controlled by LH alone, and this hypothesis is interesting in connection with the findings of Wilson & Sharp (1976a, 1976b) that oestrogen injections given to hens at suitable times do not provoke LH surges, unlike progesterone and testosterone. Senior & Furr (1975) demonstrated an uptake of oestrogen by the larger ovarian follicles at between 20 and 50h before ovulation whilst their analysis of other parts of the ovary showed that the highest concentrations of oestrogens per gram of tissue were found in the stroma and the small follicles. It is tempting to suggest, therefore, that the ability of major ovarian follicles to secrete steroid hormones is a vital factor to the process of ovulation in the hen. It is interesting to note in this context that the secretion of progesterone brought about by a subcutaneous injection of 25μg LH-RH is much greater 30h after an ovulation than 6h after an ovulation (Etches & Cunningham, 1977). The ability of the ovary to produce progesterone may, therefore, be related to the maturity of the largest follicle, since at 30h after an ovulation, the largest follicle is mature and due to ovulate within the next 6 to 8 hours. In this context, maturity of the follicle refers to its steroidogenic capability and not to its weight.

2.4.5 Neural Involvement in the Control of Hormone Secretion

Rothchild (1949) was the first to suggest that progesterone does not induce ovulation by acting directly on the pituitary and implied it acted directly on the brain. He found that progesterone placed in the pituitary was no more effective than if applied to distal sites such as thigh muscle. Firm evidence of this suggestion was obtained by Ralph & Fraps (1960). These authors found

that 5 or 10μg implants of progesterone in the hypothalamus caused premature ovulation whereas implants of similar quantities of the hormone in the pituitary had no effect. Ralph & Fraps (1959) have also shown that ovulation can be interrupted by lesions in the para-ventricular nucleus of the hypothalamus, while progesterone positive feedback is abolished by ablation of the pre-optic region. Further evidence of neural involvement has come from the use of barbiturate drugs. Fraps & Case (1953) found that Dial (5:5 diallylbarbituric acid), Ipral calcium (probarbital calcium) and Nembutal (pentobarbital sodium) could induce ovulation in a small but significant number of injected hens. Moreover, Dial and Nembutal tended to enhance the ovulation inducing properties of progesterone injected at low doses (0.1 mg/hen). Yet another barbiturate, phenobarbital sodium never induced premature ovulations and was found to supress the ovulation of the second, but not the first follicle of a sequence when injected up to 9h before the expected event. It was also found to block the progesterone-induced premature ovulation of the first follicle of a sequence if the steroid was given between 30 and 40 minutes following barbiturate administration (Fraps, 1955). These findings considered alongside the evidence for the existence of LH-RH in the hen reviewed above, strongly suggest a neural involvement in the ovulatory process. During the ovulatory cycle it is likely that progesterone directly or indirectly stimulates the activity of LH-RH neurones which then stimulate LH release.

2.4.6 Hypotheses for the Control of the Ovulatory Cycle

2.4.6.1 Bastian & Zarrow's Hypothesis

Bastian & Zarrow advanced this hypothesis in 1955. Recent

work has revealed its shortcomings, but the concepts it utilises are nevertheless interesting. This hypothesis assumed that the ovulation stimulus was restricted to the dark period. This stimulus, equated rather vaguely to increased LH plasma levels, was thought to be present for several hours each night. The 24 hour periodicity of this rhythm interacted with a greater than 24h rhythm of follicular maturation. Thus, follicles in a sequence would be ovulated at progressively later intervals during the dark period. At the end of a sequence, the ovulation stimulus would be present during the dark period, but the follicle next destined to ovulate would not be sufficiently mature and had to wait, therefore, for the succeeding dark period. Bastian & Zarrow recognized that this hypothesis would not account for lag since, if the follicles matured at a constant rate, the delayed follicle would ovulate at the beginning of the open period and the succeeding follicle, being similarly mature, would ovulate at exactly the same time the following day. The hypothesis therefore included a concept of graded follicular maturity whereby a follicle of slightly less than full maturity could be ovulated if the ovulatory stimulus was present for a sufficient time. Bastian & Zarrow (1955) realised that this could imply that the second and subsequent eggs of a sequence would have smaller yolks than the first, and showed that this was true for short sequences. The authors were also aware that maturity of a follicle, in the sense of having the capacity to ovulate, does not correlate well with maturity in terms of follicular growth. The chief difficulty presented by this hypothesis is the failure of subsequent workers measuring plasma LH by RIA to demonstrate a peak, or prolonged, increased levels, during the dark period on the day of missed ovulation (Furr et al., 1973; Wilson &

Sharp, 1973). Another drawback of this hypothesis is its failure to take account of positive feedback, although this is a less serious objection.

2.4.6.2 Frap's hypothesis

This hypothesis was put forward by Fraps in 1955. Here emphasis was laid on a neural basis of diurnal periodicity controlling ovulation-inducing hormone (LH) release. The neural basis was envisaged as a diurnal variation in the threshold of response to an excitation hormone, presumed to be progesterone. This can be alternatively expressed as a diurnal variation in the response of the brain to progesterone-induced positive feedback. Fraps (1955) divided the ovulatory cycle into an open period and a closed period. The open period lasts about 8 or 9h (under 14L:10D conditions) during which the pre-ovulatory secretion of LH is initiated and the neural threshold is low. This threshold is, however, high for the remaining 15 or so hours of the closed period of the cycle. The maturing follicle was presumed to secrete increasing quantities of excitation hormone until the response threshold was surpassed resulting in LH secretion and, subsequently, ovulation. As follicles mature at approximately 26h intervals, each pre-ovulatory release of LH and its associated ovulation were considered to occur later each day by the period of lag. Eventually, a follicle would mature during the closed period. Then, LH release and ovulation would not occur and a pause day would result because the follicle must wait for the next open period. An obvious test of this hypothesis would be to verify if the plasma levels of progesterone, the presumed excitation hormone, rise on the day of missed ovulation. No evidence for this has been found to date (Peterson & Common, 1971; Kappauf & van Tienhoven, 1972; Haynes, Cooper & Kay, 1973; Furr et al.,

1973). Neither is testosterone, which can also induce LH secretion in the hen (Wilson & Sharp, 1976a), secreted in increased quantities on the day of missed ovulation (Peterson, Henneberry & Common, 1973; Etches & Cunningham, 1977).

2.5 Factors Involved in Sexual Maturation and Lay

This discussion is concerned with two important factors: the effects of hormones, and the effects of light. It is important to note, however, that the age at sexual maturation differs between strains of hen. Waters (1934) noted that lightweight breeds such as the Leghorn mature earlier than heavier breeds. To what extent this is due to the digestion and assimilation of nutrients, or to the interaction of the mechanisms controlling the secretion of growth hormone and gonadotrophins from the pituitary is not clear. It is interesting to note that the onset of puberty can be delayed by poor nutrition, however, and poorly-nourished birds will start to lay at lower body-weights than well-nourished birds (Prentice, Baskett & Robertson, 1930).

2.5.1 Endocrine factors

2.5.1.1 Hormone profiles

A series of phenotypic changes are seen in the maturing hen. Body weight increases steadily until around the first week of lay (21st - 25th weeks of age). At the 16th to 19th week (under standard 14L:10D conditions) the comb starts to redden and grow rapidly, and the cloacal vent enlarges and swells shortly before the first egg is laid. Plasma LH concentrations rise during the first week after hatch. After this initial maximum, LH levels in the blood decline slightly, then remain constant until 16 - 19 weeks of age when a secondary rise is

observed. Plasma LH levels fall from this pre-pubertal peak over a two to three week period, following which egg-laying commences. These data, reported by Sharp (1975) were compared with the period of rapid comb growth and it was observed that rapid comb growth is closely related to the duration of the prepubertal LH peak. Wilson & Sharp (1975c) have shown that the onset of ovarian follicular development occurs at the time of the pre-pubertal rise in plasma LH concentrations.

Development of the secondary sexual characters such as comb growth, and the development of the oviduct are known to be regulated by gonadal steroids (Parkes & Marshall, 1960). Direct measurements of circulating oestrogens have shown that plasma concentrations of these hormones increase, as would be predicted, at 6 - 8 weeks before the onset of lay (Peterson & Webster, 1974; Senior, 1974). It is not clear however, if this pre-pubertal increase precedes, parallels or post-dates the pre-pubertal increase in LH secretion.

2.5.1.2 Development of the control of LH secretion

Immature pullets with undeveloped sexual organs do not respond to a standard dose of progesterone by increasing the secretion of LH in the way that laying hens do (Wilson & Sharp, 1975c). This positive feedback response develops during the 9 weeks before puberty at the time when the ovary, oviduct and comb start to grow rapidly. The changes in the sensitivity of the pituitary to synthetic LHRH during sexual maturation have also been documented (Wilson & Sharp, 1975c). Sexually undeveloped pullets and pullets in the early stages of sexual development showing a weak LH positive feedback response to progesterone responded in an identical way to a standard dose of LHRH. As development proceeded, the responses to standard doses of LHRH and progesterone diminished, so that 3 - 4 weeks before the onset of lay, these treatments

resulted in a small increase in LH secretion. One or two weeks before the onset of lay, at the time when yellow yolk follicles develop in the ovary, a further fall in pituitary sensitivity to LHRH was noted, but injected progesterone resulted in a prolonged increase in LH secretion. Evidently, two characteristics of the pre-pubertal period in the hen are a progressive decrease in the sensitivity of the pituitary to LH-RH and an increase in the sensitivity of the brain to the positive feedback action of progesterone.

It has been proposed that a decrease in the sensitivity of the negative feedback system controlling gonadotrophin release is the initiator of sexual development (review: van der Werff Ten Bosch & Donovan, 1965). There is, however, a difficulty in applying this hypothesis to explain the pre-pubertal rise in plasma LH in the hen: abolition of negative feedback by ovariectomy increases mean plasma LH levels in hens of 8 to 12 weeks of age, but a secondary pre-pubertal rise is still observed in these animals (Sharp, 1973). Perhaps another unidentified factor causes increased LHRH stimulation of the pituitary at this stage of development.

Additionally, it is not known why plasma LH should decline from a pre-pubertal maximum during the 3 to 4 weeks before the onset of lay in intact hens. In the cockerel, LH plasma levels rise during sexual development, but there is no decline before or after the first sperm are produced (Sharp, 1975). This suggests that ovarian steroids are more effective in suppressing LH secretion and are secreted in increasing quantities in the final stages of maturity. Senior (1974) has shown that concentrations of oestradiol are approximately twice as high in laying hens as in young, immature hens. It would be valuable to measure progesterone levels in growing fowl to see if

increasing quantities of this hormone are found in the blood at the stage of sexual development when plasma LH levels are falling from the pre-pubertal maximum, as postulated by Sharp (1975). This suggestion is reasonable as progesterone will depress plasma LH levels in the ovariectomized hen and is particularly effective in combination with oestrogen (Wilson & Sharp, 1976b).

2.5.2 Light

Much experimental work has been done as the effects of light on growth and sexual maturity of the fowl (Morris, 1967). This author concluded that the effect of light on growth rate is due to the pattern of activity and hence food intake induced by different periods of lighting, hence maximum growth is induced by using lighting schedules which encourage chicks to feed at any time of the day or night. Two simple schedules of this kind are continuous light or continuous dark. Morris (1967) has also emphasized how the photoperiod can affect the rate of sexual maturity and the rate of egg production. Also, it seems that chickens are more affected by changes in the length of the photoperiod at any one time. In an experiment conducted by Morris (1962) pullets reared under a 22h photoperiod matured one week earlier than pullets reared under a 6h photoperiod, and the maximum rate of sexual maturation under constant photoperiods could, in fact, be achieved with just 10h light daily. If, however, decreasing photoperiods were used during rearing, the mean age of sexual maturity was delayed while the reverse treatment (increasing photoperiods) advanced sexual maturity by some 33 days. Morris (1967) has pointed out that flocks which mature very quickly usually give poor subsequent egg yields, and to maximize egg yield, it is better to aim for a 'middle' value for sexual maturity.

No information is available relating these different photoperiodic treatments to the endocrine variables affecting reproductive performance.

2.6 Factors Involved in the Cessation of Lay and the Decline in Reproductive Activity

2.6.1 Seasonal factors

Many years ago, commercial flocks of hens were kept under natural environmental conditions and it was commonplace for flocks to suffer a marked reduction in egg production around the end of October. Egg production remained low in November and December and started to increase towards the end of January. This seasonal pattern of laying gave rise to the terms 'first laying year' and 'second laying year' and in such cases the laying year was synchronised with the calendar year, although in modern usage the first laying year is generally taken to mean the 12 egg-production months following the onset of lay. The term biological laying year, first used by Zander et al. (1942), refers to the period of time between the first egg laid and the last egg before the annual rest, in the case of the first year. In subsequent years, the biological laying year extends from the first egg laid at the resumption of production to the next annual rest. Zander et al. (1942) have shown that the biological laying year in White Leghorns is longer (mean length = 384.6 days) than the calendar year, while Hays (1943) recorded a figure of 377 days for Rhode Island Red pullets. At the end of the biological laying year, no eggs are laid for about 3 months while the hen moults (Lerner & Taylor, 1941). It may readily be seen that this period of cessation of lay, termed the annual rest, is not coincident with the reduced rate of lay in winter months observed in

flocks of hens kept under natural environmental conditions. Whetham (1933) has shown that rates of lay decline in the winter in flocks kept at mid ($40^{o}N$) latitudes, but not at near-equatorial latitudes. This suggests that short winter photoperiods in the Northern Hemisphere affect rates of lay in hens kept outdoors.

2.6.2 Preventative effects of a constant environment

It was shown by Greenwood (1962) that egg production could be greatly increased by the use of a constant length artificial photoperiod coupled with stable hen-house temperatures in the winter months. The chief benefit was the elimination of falling rates of production in the winter months but the length of the biological laying year was also increased. This ranged from 13 months to 24 months, and Greenwood (1962) also noted a reduction in the mean length of the annual rest from 107 days (control birds, natural environment with supplementary winter lighting) to 32 days in the experimental birds.

Evidently, the mean rate of egg production of hens kept in a constant environment will decline in the second calendar laying year since individual birds will cease production and moult at different times, and for different periods throughout the year. It would be interesting to obtain an estimation of the incidence of the annual rest in the second laying year to assess its importance as a factor contributing to declining egg production in modern strains of hen. It is also evident from the data of Zander *et al.* (1942) that a hen's egg production at the beginning of a subsequent biological laying year is higher than at the end of the previous biological laying year. If hens are forced to rest by food and water deprivation before the end of the laying year, they quickly regain production after the experimental

treatment and lay at much higher rates than control birds (Len, Abplanalp & Johnson, 1964). Information collected by ADAS (Ministry of Agriculture's Agricultural Development and Advisory Service) reveals that a rest can be induced by a variety of methods such as drug administration, food and/or water deprivation, low calcium or low sodium diet. The beneficial after-effect appears to be independent of the method used to induce the rest. It seems that the benefit gained is due to the period of inactivity imposed on the reproductive system. Why this is so remains uncertain.

2.6.3 Ageing and reproduction

2.6.3.1 Ageing and Egg Laying Patterns

Zander et al. (1942) hypothesized that senescence was manifested as a decrease in length of the biological laying year, and an overall decrease in the rate of lay in each successive biological laying year. The former phenomenon would contribute to lower rates of lay for flocks in the third calendar year of production, but an overall decrease in the rate of lay is noticeable, even in the first calendar year (part of the first biological laying year) of egg production. ADAS egg production figures (personal communication, 1977) derived from the performance of many commercial flocks support this contention. Flocks tend to reach peak rates of lay in the second or third month following the onset of lay, but the rate declines steadily thereafter, and is more marked in some strains (e.g. broilers) than others (see p. 1). It seems, in view of the evidence reviewed above, that this decline in flock rates of lay reflects a decline in rates of lay in all individuals in the flock rather than individual birds entering a rest period. However, only a detailed examination of individual laying records could

verify this. Also, it appears to follow from Zander & co-worker's (1942) definition of senescence that broiler type hens may age more quickly than egg-type hens since the decrease in the rate of lay is much steeper in the broiler type birds.

2.6.3.2 Possible causes of age-linked ovulatory failure

As discussed earlier, ovulation depends on the functional integrity of the hypothalamo-pituitary-ovarian axis. Thus, failure to ovulate could, in theory, occur as a result of a failure at any point on this axis. If the hypothalamus becomes insensitive to positive feedback, or insufficient LH-RH is produced, then ovulation would not occur. Similarly, the pituitary might lose its responsiveness to LH-RH or its capacity to synthesize LH. The ovary may fail to respond to LH or it may produce insufficient progesterone. Lastly, it may simply exhaust its stock of oocytes.

This last suggestion may be dismissed as Zander et al. (1942) calculated that the number of oocytes developing into ova was not limiting with age. From estimates of the total number of eggs laid in the lifetime of a hen, it has been calculated that only 20% of the follicles observed in a 15-day old chicken ovary ever reach maturity (Fauré-Fremiet & Kaufmann, 1928). Studies on oocyte numbers in other species (review: Jones, 1975) indicate that the exhaustion of the oocyte stock is not the primary age lesion in rodents. In contrast to this however the CBA strain of mouse and the human do show ovarian failure in old age. Jones (1975) has nonetheless acknowledged that the oocyte population may decline with age in many species but this loss is accompanied by alterations in the functional capacity of the pituitary which may be due, in turn, to impairment of the hypo-

thalamic controlling mechanisms. Good evidence for the ovary not being the primary site of the failure of reproductive actitivity in aged female rats has been obtained by Krohn (1962) who found that ovaries transplanted from anoestrous females to young, ovariectomized females enabled the recipients to resume regular oestrous cycles. Most investigations into reproduction and ageing have been performed on rats, and there is now a body of evidence pointing to a primary age lesion in the hypothalamus in this rodent. Huang and Meites (1975) succeeded in reversing the early symptoms of reproductive ageing (constant oestrous) by injecting L-dopa twice daily. Labhsetwar (1970) has showed that there are increased pituitary stores of FSH and LH in aged rats, indicative of reduced LH-RH secretion, a suggestion which Jones (1975) supports. An interesting hypothesis advanced by Pi et al. (1973) suggests that the FSH-RH mechanism remains intact with age, but that the LH-RH mechanism becomes defective. The existence of FSH-RH is debatable, however, and this hypothesis must be regarded as controversial.

It remains to be established whether age-linked changes in the egg-production rate in hens directly reflect changes in the ovulation rate. If so, where is the primary age lesions in the chicken? The widespread availability of hormones, which can be injected to test the sensitivity of each of the component organs of the hypothalamo-pituitary-ovarian axis, and the existence of sensitive RIA to measure responses mean that this problem is very amenable to experimental investigation.

2.7 Objectives

This study was instituted with the object of elucidating the

causes of the declining rate of lay in the hen's first twelve months of egg production. As pointed out previously, this decline is more marked in the broiler parent strains, and presents a problem to the poultry industry. One of the first questions to be asked was: What are the hormonal factors responsible for ovulation? Failure of the endocrine mechanism controlling ovulation may be the primary cause of the fall-of in egg production with age. Studies were therefore carried out on the hormonal changes occurring at the onset of lay and during the ovulatory cycle.

Further consideration of the problem resulted in investigations into egg-laying differences attributable to strain, and investigations into differences attributable to age. Thus, the structure of the ovary and the phase of rapid development of yellow yolk follicles were investigated with reference to the variables of age and strain. Individual egg laying records of three strains of hen covering the first twenty-two months of production were also examined, partly to establish precise egg production data for other experiments and partly to assess the importance of factors such as the annual rest and sequence length to the problem of declining rates of lay. The times of lay within a sequence were also investigated as previous experiments (Heywang, 1938; Fraps, 1955) were conducted using strains of hen which are now obsolete.

Work, reviewed above, on ageing and reproduction in the rodent stimulated the design of a series of experiments aimed at identifying the primary age lesion in the hen's reproductive system. It was planned to test the sensitivity of each component of the hypothalamo-pituitary-ovarian axis, using an appropriate hormonal challenge, in peak of lay and older hens.

3. MATERIALS & METHODS

3. MATERIALS & METHODS

3.1 Animals, housing and husbandry

3.1.1. Hens

Three strains of hen were used: Ross I, a heavyweight broiler breeder; Ross Ranger, a mid-weight brown egg producer; Babcock B300, a lightweight white egg producer. Day-old chicks were purchased from commercial breeders on 3rd September 1974 and reared on a 14 h daily photoperiod in brooders at a temperature of 25°C. Food (chick starter mash, SAI Ltd) and water were provided ad libitum.

At eight weeks of age, birds were transferred to a commercial-type windowless hen-house (14 h photoperiod; minimum temperature 17°C). They were caged individually and fed either SAI layers mash or pellets. Full access to food and water was allowed except in the case of the Ross I pullets which were fed a restricted diet (Blair, MacLowan & Bolton, 1976), in accordance with current commercial practice.

300 hens of each strain were initially reared and maintained in this manner at the Agricultural Research Council's Poultry Research Centre (PRC) outstation at Roslin. A further 100 Ross I and 100 Babcock B300 chicks were purchased from a commercial breeder on 31st October 1975 and were reared and managed as described above.

For some experiments, small groups (20 - 30) of laying hens were brought to the main Research Centre at King's Buildings, Edinburgh where housing and management were identical to the conditions at Roslin. Some hens were put out of lay temporarily by this removal, and such hens were not selected for experimental purposes.

PRC hens derived from Shaver stock (white leghorn type) hatched and reared at Roslin, and maintained at King's Buildings as described above, were used for a detailed study of laying patterns.

3.1.2. Grouse

Female red grouse (Lagopus lagopus scoticus) were reared outdoors in cages built on moorland adjacent to the Institute of Terrestrial Ecology's Research Station at Blackhall, Grampian. During rearing, birds were exposed to natural environmental light and temperature and ate mostly heather shoots. On completion of rearing, birds were housed in large, outdoor cages at Blackhall and given free access to water and a special pellet diet formulated for game birds. This diet was supplemented with heather shoots.

3.1.3. Bantam Hens

Ten bantam hens and one cockerel were obtained from the Institute of Terrestrial Ecology's Research Station at Blackhall as adults (aged less than 1 year). They were housed together in one large floor pen with individual nest boxes provided for each hen. A 14h photoperiod was used, and the birds were allowed free access to food (SAI layers mash) and water.

3.2 Compilation of Laying Records

Eggs were collected twice daily at Roslin and records were kept for each bird. Records for 23 laying months (one laying month = 28 days) were transferred to punch cards for computer analysis.

The times of oviposition for each hen housed at King's Buildings were recorded to the nearest minute. All cages were fitted with a device that detected the passage of an egg rolling out of the cage. Information from these sensors was passed to an electronic time storage bin which accumulated information and transferred it every minute onto paper tape.

Tape was interpreted with either a teletype or a computer programm. Manual records were maintained as a check on this system.

3.3. Collection of blood samples

Syringes, $2\frac{1}{2}$ or 5 ml, were rinsed with heparin, 5,000 i.u./ml (Pularin, Evans Medical) before use. Normally 25 gauge $\frac{5}{8}$" or 1" needles were used, although 23 gauge needles were sometimes used for the heavier hens. The needle was bent at an angle of about 30^{o} to the shank about half-way along its length and attached to the syringe hub. Blood samples were taken from the brachial vein which was exposed by plucking feathers from the inferior surface of the wing. 70% alcohol was swabbed over the epidermis to clarify it and skin tension was applied at 90^{o} to the course of the vein before puncture, and withdrawal of blood. Normally, light pressure applied distal to the sampling site for 10 - 20 seconds after needle withdrawal arrested haemorrhage. Up to 6 serial samples at 20 minute intervals could be taken from one brachial vein using this technique, and blood could be withdrawn within 2 minutes of removing a bird from its cage.

Plasma was separated by centrifugation and stored in stoppered vials at -20^{o}C until required for assay.

3.4 Radioimmunoassay Methods

3.4.1 Luteinizing Hormone (LH)

LH was measured by radioimmunoassay (RIA) as described by Follett *et al.*, (1972) using a different antiserum, raised in rabbits against a partially purified LH preparation, designated α CM2 15/8 and used at a 1 : 20,000 dilution.

In experiments where multiple hormone measurements were made from a limited volume of plasma, the method was modified as follows:

The sample volume of standard and plasma samples was 50µl. Antiserum IRC2/T (1: 1500 dilution) was added in 50µl aliquots, and 50µl aliquots of ^{125}I - LH were used. Donkey anti-rabbit serum was diluted to 1: 200 using 1: 200 normal rabbit serum. Otherwise, the method was identical to that of Follett et al., (1972).

3.4.2 Progesterone

Initially, progesterone was measured by radioimmunoassay as described by Scaramuzzi et al., (1975). This method involves the use of dextran-coated charcoal to separate free and bound steroid and has the disadvantage that the dextran-coated charcoal is liable to absorb a proportion of the steroid bound to antiserum as well as the free steroid. To compensate for this, standards, control and unknown plasmas have to be incubated according to a strict time and temperature protocol. In practice, this is achieved using small assays (not more than 100 tubes) so that little time lapses between addition of dextran-coated charcoal to the first and last tubes. The incubation is stopped si multaneously by centrifugation. The disadvantage of small assays is that plasmas from one experiment cannot always be assayed together. Comparison of potencies of unknown samples is therefore complicated by error due to between-assay variation.

This problem was overcome by using a solid phase radioimmunoassay described by Dighe & Hunter (1974) in which the antiserum was linked covalently to microcrystalline cellulose. Preliminary experiments using Dighe & Hunter's antiserum yielded unsatisfactory results unless assay tubes were rigorously washed in double glass distilled deionised water and the use of plastic tubing in dispensing was avoided. By substituting Dighe & Hunter's antiserum with one raised by Scaramuzzi et al., (1975) the assay was found to work satisfactorily without having to pre-wash the assay tubes.

The antiserum raised by Scaramuzzi et al., was therefore found to be more convenient for routine use.

3.4.2.1 Preparation of Standards

Progesterone (Sigma Co. Ltd.) was first prepared as a 1.28 mg/ml solution in ethyl alcohol (Analar, BDH). From this, a 12.8 µg/ml ethanolic solution was prepared, and the concentration was adjusted using an estimate obtained on a Pye Unicam SP600 ultra-violet spectrophotometer (λ = 240 nm; slit width .2 mm). From this, 10 ml aliquots of six standard dilutions covering the range 12.8 µg/ml to 0.4 ng/ml were prepared. All standard solutions were stoppered and stored at 4°C. These solutions could be reliably stored for one month.

3.4.2.2 Preparation of Standard curve

Triplicate 0.1 ml aliquots of each standard in thin wall glass tubes, 75 x 16 mm, were prepared, covering the range 1280 - 40 pg/tube. In addition, triplicate 0.1 ml aliquots of 12.8 µg/ml solution (1280 µg/tube) were prepared as non-specific binding tubes.

Tubes were dried under air at 50°C for 10 minutes, removed and laid out in a rack with six untreated tubes serving as zero standards.

Additional triplicate batches containing 80 pg/tube, 320pg /tube and 640 pg/tube were prepared to serve as internal standards for estimating recovery after extraction.

3.4.2.3 Extraction of Progesterone from plasma

Plasma (0.1 or 0.2 ml) was pipetted into unstoppered 75 x 16 mm thin wall glass tubes. Ethanol (10 or 20 µl) was added. Tubes were mixed and allowed to equilibrate for 15 mins. Plasma added to internal standard tubes was mixed and allowed to equilibrate for 15 mins before the addition of ethanol and subsequent equilibration.

Each tube was extracted twice with redistilled light

petroleum, b.p. 40 - 60°C (Analar, BDH) in the following manner. Light petroleum (10 vol : 1 vol. plasma) was added to all tubes, including a set of empty tubes serving as controls for a solvent blank effect. These were extracted for 3 minutes on a multivortex mixer (Baird & Tatlock Ltd); thereafter, tubes were transferred to a mixture of dry ice and methanol to freeze the aqueous phase. The organic phase was poured off into a fresh glass tube. Both extractions were pooled, and the organic phase was evaporated under air at 60°C for 15 minutes.

Six replicates of a quality control pool of laying hen plasma were run in each assay to allow an estimate of interassay variance to be made. Quality control plasma was also added to the internal standard tubes prior to extraction.

3.4.2.4 Routine assay procedure

1, 2, 6, 7 - ^{3}H (n) progesterone (Radiochemical Centre, Amersham) was dissolved in phosphate buffered saline (0.05M, pH7.0), to which 1% w/v gelatin had been added, to produce a solution containing 10 pg/ml of the tritiated steroid. Sucrose (Analar, BDH; 15 g/100 ml) was added to this solution and dissolved by stirring. A sucrose gradient was used to prevent the microcrystalline cellulose to which the antiserum was linked from settling out too quickly. 1 ml aliquots were added to 3 assay tubes (total count tubes). To the main solution was added sufficient antiserum to produce optimum assay dilution (see p.42). The solution was mixed well, and 1 ml aliquots were added to the remaining assay tubes. Following this, each tube was mixed vigorously on a whirlimixer (Fisons Ltd) for 10 seconds. Tubes were incubated overnight at 4°C following which they were centrifuged at 3300 r.p.m. for 40 minutes at 4°C (MSE mistral 6L centrifuge). The supernatants were decanted into glass scintillation vials. 8 ml of

scintillant composed of 4 g PPO (Koch-Light Laboratories) per litre sulphur-free toluene (BDH Ltd) was added to each vial which was then stoppered and mixed well. Vials were left for at least one hour in the dark at 4°C before the unbound radioactive tracer was measured on either a Philips or a Nuclear Enterprises liquid scintillation counter. Output from the scintillation counter was obtained as punched tape.

3.4.2.5 Data Analysis

Assay data were analysed using a computer program written in Fortran IV and run on an IBM 1130 computer. The program, written by Mr D Maxwell and Dr D Sales of the Agricultural Research Council's Animal Breeding Research Organisation, Edinburgh used an iterative least squares weighted regression technique to produce a logit/log plot of the standard dose/response calibration curve, from which unknown potencies together with 95% confidence limits were estimated. Maxwell & Sales' program is based on that described by Rodbard & Lewald (1964) but all statistical subroutines have been modified to improve the accuracy of the potency estimates and the quality control parameters, which are features of the original program.

For steroid assays, an additional package was developed which calculated the regression line for the series of internal standards and the quality control plasma run in each assay, using an iterative least squares weighted regression technique. Taking the slope of this line as the recovery factor for all unknown plasmas in the assay, unknown potencies were adjusted to take account of losses incurred in the extraction procedure.

3.4.3 Preparation of antiserum

3.4.3.1 Activation of cellulose

20 g cyanogen bromide (BDH Ltd), 20 g cellulose microcrystalline (E Merck Ltd) and 100 ml distilled water were mixed in a

2 litre reaction vessel. The mixture was stirred and 2M NaOH was added until the pH did not drop below 11.0 If necessary, ice granules were added to prevent the reaction overheating. The liquor was filtered at the pump using a coarse glass sinter, and washed with the following series of liquids: 2 litres 0.1M Sodium Bicarbonate; 500 ml 50% acetone; 500 ml 70% acetone; 1 litre 100% acetone. The cellulose was dried and stored in a desiccator at -20°C until required for use.

3.4.3.2 Linking antiserum

1 g activated cellulose was suspended in 4 ml 0.1M sodium bicarbonate solution. 200µl antiserum was added and the solution was stirred in a closed container at room temperature for 24 h. This mixture was centrifuged using a bench centrifuge and the precipitate was washed five times with 10 ml 0.1M sodium bicarbonate solution, centrifuging between washes and discarding the supernatant. The precipitate was then washed with 50 ml 0.2M acetate buffer (pH4.0), centrifuged, and the supernatant was discarded. The precipitate was resuspended in 10 ml acetate buffer and sonicated for 20 seconds. This mixture was left stirring overnight. After centrifugation, the supernatant was discarded. Following this, two more washes with sodium bicarbonate solution, and four washes with 0.05M phosphate buffered saline were performed as described above. The linked antiserum was then resuspended in 20 ml phosphate buffered saline to produce a nominal 1 : 100 suspension, and stored at 4°C until required for use.

3.4.4 Tests on antiserum

3.4.4.1 Dilution tests

Triplicate total count tubes and a series of zero standards all containing 10 pg tracer / 1 ml assay diluent were prepared. Each

set of zero standards contained a different antiserum concentration, starting with 1 : 1000 and proceeding via doubling dilution to 1 : 128000. Tubes were treated according to the standard assay protocol. From the radioactivity counts obtained, a % tracer bound vs. dilution was constructed. The working dilution of antiserum was chosen arbitrarily as that which would bind 60 - 70% labelled antigen in the absence of unlabelled antigen.

3.4.4.2 Parallelism of bound and free antisera

Sets of standards were treated according to the assay protocol for the solid phase antiserum, and according to Scaramuzzi et al., (1975) for the unlinked antisera. The gradients of the log / logit dose response curves were obtained and compared (Table 1).

3.4.4.3 Specificity tests

The assay described by Scaramuzzi et al., (1975) used serum from the ninth bleeding of sheep FD91920 injected with progesterone 11 - hemisuccinate linked to bovine serum albumen. In the work described in this thesis, bleed 10 from the same animal was used, and tests for all cross-reacting steroids were made for all compounds which showed a cross-reaction of greater than 1% with bleed 9 antiserum. These tests were achieved by preparing an appropriate range of dilutions of the cross-reacting steroids and then assaying them following the standard assay protocol. The quantities of these steroids at the 50% bound (with reference to zero standards = 100% bound) region of the dose response curve were compared to the quantity of progesterone bound at that point to provide an estimate of cross-reaction (Table 2).

Table 1. Comparison of Assay Variables (Means $\pm$ S.D)

	Solid - Phase Assay (20 examples)	Dextran Charcoal Assay (15 examples)
Gradient	-0.91 ± 0.1	-0.93 ± 0.22
50% intercept	4.37 ± 1.12 ng/ml	3.70 ± 1.8 ng/ml
Between assay variance	14.07 ± 1.03 %	13.18 ± 1.47 %
Within assay variance	3.59 ± 1.43 %	3.11 ± 2.53 %

Table 2. Cross-reactions of Bleeds 9 (Data obtained from Scaramuzzi et al; 1975) and 10 of sheep FD91920 injected with Progesterone 11 - hemisuccinate - BSA

Compound	Bleed 9	Bleed 10
Progesterone	100	100
20α - dihydroprogesterone	1.1	1
11β - hydroxyprogesterone	77.5	145.5
11α - hydroxyprogesterone	121	160
Cholesterol	< 0.05	< 0.01
Pregnenolone	0.9	0.01
5β Pregnane 3,20-dione	9.8	11.2
5α Pregnane 3,20-dione	5.2	2.6
17α Hydroxyprogesterone	2.2	2.0
11 Ketoprogesterone	82.4	35.0

4 RESULTS & DISCUSSION

The significance of progesterone in reproductive activity

Progesterone has different roles in mammals and birds. In the hen, progesterone is the major steroid responsible for inducing the pre-ovulatory LH surge, (p. 16) while oestrogen performs this role in mammals (Ferin et al., 1969; Goding et al., 1969; Scaramuzzi et al., 1970; Brown - Grant, 1969; Dierschke et al., 1973; Yen & Tsai, 1972). The two main functions of progesterone in the mammal are generally considered to be the maintenance of the uterus in a suitable condition for implantation of the embryo, and to prevent the development of further ova by suppressing FSH secretion (Brown - Grant, 1969). In mammals, the chief source of progesterone is the corpus luteum which develops from the follicle wall after ovulation, but no analagous structure is found in birds and the post-ovulatory follicle is rapidly resorbed (Deol, 1955; Van Tienhoven, 1959). One may, therefore suggest that further studies on the role of progesterone in inducing the pre-ovulatory surge and additional observations on plasma progesterone levels in mature non-laying birds could be helpful in clarifying its significance in avian reproductive activity.

4.1 Variations in Blood Levels of LH and Progesterone during a Reproductive Cycle of Egg Laying, Incubation and Brooding in Bantam Hens

The breeding cycle of the hen can be divided into three distinct stages: a laying period, which terminates when the hen has accumulated a clutch of eggs, an incubation period of 21 d and a brooding period during which the hen exhibits maternal behaviour towards her chicks. When the brooding period ends, the hen resumes laying providing the environmental conditions are favourable.

Just before the onset of lay, and during the laying period, the

hen's comb is red and has a shiny, waxen appearance. The incubating hen, in contrast, has a pale, shrivelled comb and loses feathers from the ventral thoracic region which then becomes highly vascularized. This vascular area is termed the brood patch. The brood patch regresses and feathers regrow during the brooding period. The hen utters a characteristic broody call when incubating and brooding.

This study was designed to determine the changes in circulating levels of LH and progesterone during a complete cycle of broodiness in the bantam. Bantam hens were chosen because they can be induced to become broody more easily than commercial egg-producing hens. A flock of nine hens were mated to one cockerel kept in the same floor pen. Some hens had come into lay just prior to the start of the experiment, but others were incubating. All hens were deprived of their eggs and the experiment was commenced. Hens were allowed to accumulate a clutch of eggs, and blood samples ($2\frac{1}{2}$ ml) were taken at twice weekly intervals in the afternoon. Notes were made of the appearance of the comb, feathering and behaviour week by week. The study was terminated when hens came back into lay. Plasmas were assayed for progesterone and LH.

The mean levels of plasma LH and progesterone during the three stages of the reproductive cycle are summarized in Table 3. LH levels dropped sharply when birds started to incubate and were generally at a nadir at the end of incubation. LH levels tended to increase throughout the brooding period and reached typical in-lay levels just before laying was resumed. Progesterone plasma concentrations tended to be higher when birds were in lay, and lower in the incubation and brooding periods. The levels of progesterone were noticeably more variable then plasma LH levels, and occasional peaks of progesterone reaching levels seen in laying hens were observed in incubating and brooding

Table 3

Plasma Concentrations of Progesterone and LH

in nine Bantam Hens in Lay,

Incubating and Brooding (Mean $\pm$ S.E.M.)

Numbers of observations are shown in parentheses

	LH ng/ml plasma	Progesterone ng/ml plasma
Laying	2.21 ± 0.07^{a} (97)	0.47 ± 0.05^{a}
Incubating	0.83 ± 0.04^{b} (40)	0.21 ± 0.03^{b}
Brooding	1.19 ± 0.05^{c} (103)	0.14 ± 0.02^{b}

Means followed by different superscripts differ significantly ($P < .001$; unpaired t-test)

Table 4

The concentrations of luteinizing hormone in the peripheral plasma of bantam hens throughout the breeding cycle (ng/ml plasma)

Time of year

Hen No.	May					June								July											August							
	14	18	21	25	28	1	4	8	11	15	18	23	25	29	2	6	9	13	16	20	23	27	30	3	6	10	13	17	20	24	27	31
1		L ←	→ L	S ←	→ S							B	→												B	L →						
		1.2	2.1	1.6	2.7	1.8	1.0	1.1	1.0	1.1	1.0	0.8	1.1	1.6	1.4	1.2	1.0	1.15	1.2	1.3	1.1	1.4	1.3	2.6	2.4	2.3	1.4	2.7	1.6	2.3	2.6	2.4	2.4
2				L ←	→ L	S ←	→ S						B	→ R							L →												
		2.6	3.0	2.6	4.1	3.8	3.5	4.2	1.1	1.2	1.2	1.4	1.0	1.5	1.2	1.3	1.2	1.1	1.3	1.5	1.3	2.0	2.0	3.1	4.0	3.4	3.3	3.2	1.9	2.8	2.7	2.2	2.8
3				L ←				→ L	? ←																							→ ?
		1.7	1.1	2.2	1.8	1.9	1.4	1.4	1.7	1.6	1.5	2.2	1.6	2.6	2.7	2.3	1.9	1.7	1.6	1.9	1.6	1.9	2.1	2.1	2.4	2.2	2.3	3.0	2.0	2.9	2.5	2.3	0.8
4			L ←	→ L			S ←	→ S					B	→													B	L →				
		2.5	1.1	2.9	3.3	2.5	3.5	2.6	1.6	0.8	0.8	0.7	0.9	0.8	1.1	1.0	1.5	1.3	1.3	0.9	1.0	1.5	1.3	1.1	1.1	1.1	1.3	2.1	1.5	2.4	2.2	3.1	2.6
5					L ←	→ L					S ←	→ S		B	→																	→ B
		2.0	1.8	1.5	1.2	1.1	1.9	1.5	1.7	1.4	2.2	1.3	0.6	0.4	0.5	0.5	0.6	0.7	0.6	0.8	0.8	1.6	0.9	0.9	1.4	1.7	1.4	1.3	1.4	1.8	2.4	1.9	2.0

6	L ⟷ L S ⟷ S/B ⟷ R L → 2.9 2.9 3.1 2.1 3.1 1.6 3.7 0.9 0.9 0.9 0.9 0.9 1.6 1.3 1.1 1.1 2.0 1.2 2.3 2.0 2.8 2.4 2.6 2.5 3.4 2.4 2.1 2.4 3.3 2.3 1.4 1.6
7	L ⟷ L S ⟷ S B ⟷ R L → 4.2 3.0 3.1 2.3 2.4 2.1 1.6 0.9 0.5 0.8 0.8 0.6 0.7 0.7 0.7 0.8 1.0 0.8 1.2 1.0 1.2 1.9 2.7 2.7 2.7 1.8 1.6 2.5 2.6 1.7 1.9 2.3
8	L ⟷ L S ⟷ S B ⟷ B L → 1.7 2.5 1.5 2.3 2.6 2.2 1.6 0.6 0.7 0.7 0.7 0.6 0.6 0.9 0.7 0.8 0.9 1.4 0.8 0.7 1.1 1.1 1.0 0.8 1.0 0.8 1.3 1.5 1.9 1.8 2.0 1.7
9	L ⟷ L S ⟷ S/B ⟷ B L → 2.3 0.8 2.6 2.7 1.9 2.5 1.7 1.4 0.8 0.6 0.7 0.7 0.6 0.4 0.5 0.8 0.8 0.8 1.0 1.0 1.1 1.1 1.4 1.0 1.1 1.2 1.9 1.5 1.4 2.3 1.1 1.1

R = Chicks removed

L = Laying

S = Incubating

B = Brooding

? = Possibly laying out of nest box

Table 5 The concentrations of progesterone in the peripheral plasma of bantam hens throughout the breeding cycle (ng/ml plasma)

Time of year

	May					June								July											August						
Hen No	14	18	21	25	28	1	4	8	11	15	18	23	25	29	2	6	9	13	16	20	23	27	30	3	6	10	13	17	20	24	27	31
1		L ←			→ L	S ←					→ S	B ←													→ B	L →						
	0.5	0.5	0.4	0.6	0.4	0.2	0.2	0.2	0.4	0.6	0.3	0.1	0.2	0.7	0.2	0.2	0.2	0.2	0.3	0.1	0.2	0.2	0.2	0.2	0.1	0.7	0.3	1.0	0.6	0.5	0.6	0.5
2			L ←		→ L	S ←							→ S	B ←					→ R		L →											
	0.4	0.3	1.3	0.9	0.5	0.6	1.2	0.3	0.3	0.4	0.3	0.3	0.7	0.32	0.4	0.5	0.3	0.3	0.4	0.3	0.20.3		0.1	-	0.3	0.9	0.5	0.8	0.7	0.6	0.7	0.7
3			L ←										→ L	? ←																		→ ?
	0.1	-	0.1	0.1	-	-	0.1	0.1	0.1	0.1	0.1	-	-	0.1	-	-	-	-	0.1	-	-	0.1	0.1	0.4	0.5	0.3	0.2	0.3	0.2	0.2	0.2	0.1
4			L ←		→ L				S ←				→ S	B ←													→ B		L →			
	-	0.6	0.1	0.4	0.6	0.3	0.3	0.1	-	0.1	0.1	0.1	0.2	0.2	0.1	-	0.1	0.1	0.1	0.1	0.1	-	0.1	0.1	0.1	0.1	0.1	-	0.2	0.1	0.5	3.1
5					L ←		→ L				S ←		→ S	B ←																		→ B
	-	-	0.1	0.1	0.3	1.2	0.5	0.1	0.2	0.3	0.1	-	0.8	-	-	-	-	-	-	-	-	-	-	24.4	0.1	-	-	-	-	-	-	-

6	L ⟷ L S ⟷ S/B ⟷ R L ⟶ - 0.1 0.2 0.1 0.2 0.2 0.2 - 0.1 0.1 0.1 0.1 0.1 0.1 0.1 - - - - 0.3 0.1 0.4 0.6 0.2 0.3 0.2 0.3 0.3 0.3 0.1 0.3 0.3
7	L ⟷ L S ⟷ S B ⟷ R L ⟶ - 0.2 0.4 0.5 3.4 0.5 0.3 0.1 0.2 0.1 0.2 0.1 0.1 0.2 0.1 0.1 0.1 0.1 - 0.3 - - 0.1 0.3 0.2 0.2 0.2 0.2 0.2 0.4 1.6 0.4
8	L ⟷ L S ⟷ S B ⟷ B L ⟶ - - 0.9 0.3 0.3 0.1 0.1 - - - 0.1 0.1 0.1 0.1 - 0.1 0.1 - - - 0.1 - 0.1 - - 0.1 - 0.3 0.2 0.2 0.4 0.3
9	L ⟷ L S ⟷ S/B ⟷ B L ⟶ 0.2 0.3 0.4 0.9 0.6 0.8 1.1 0.7 0.3 0.2 0.1 0.2 0.2 0.5 0.4 0.3 0.2 0.4 0.1 0.2 0.2 0.1 0.1 0.3 0.3 0.5 0.2 0.3 0.2 0.5 0.5 0.5

- = Below level of detection

L = Laying

S = Incubating

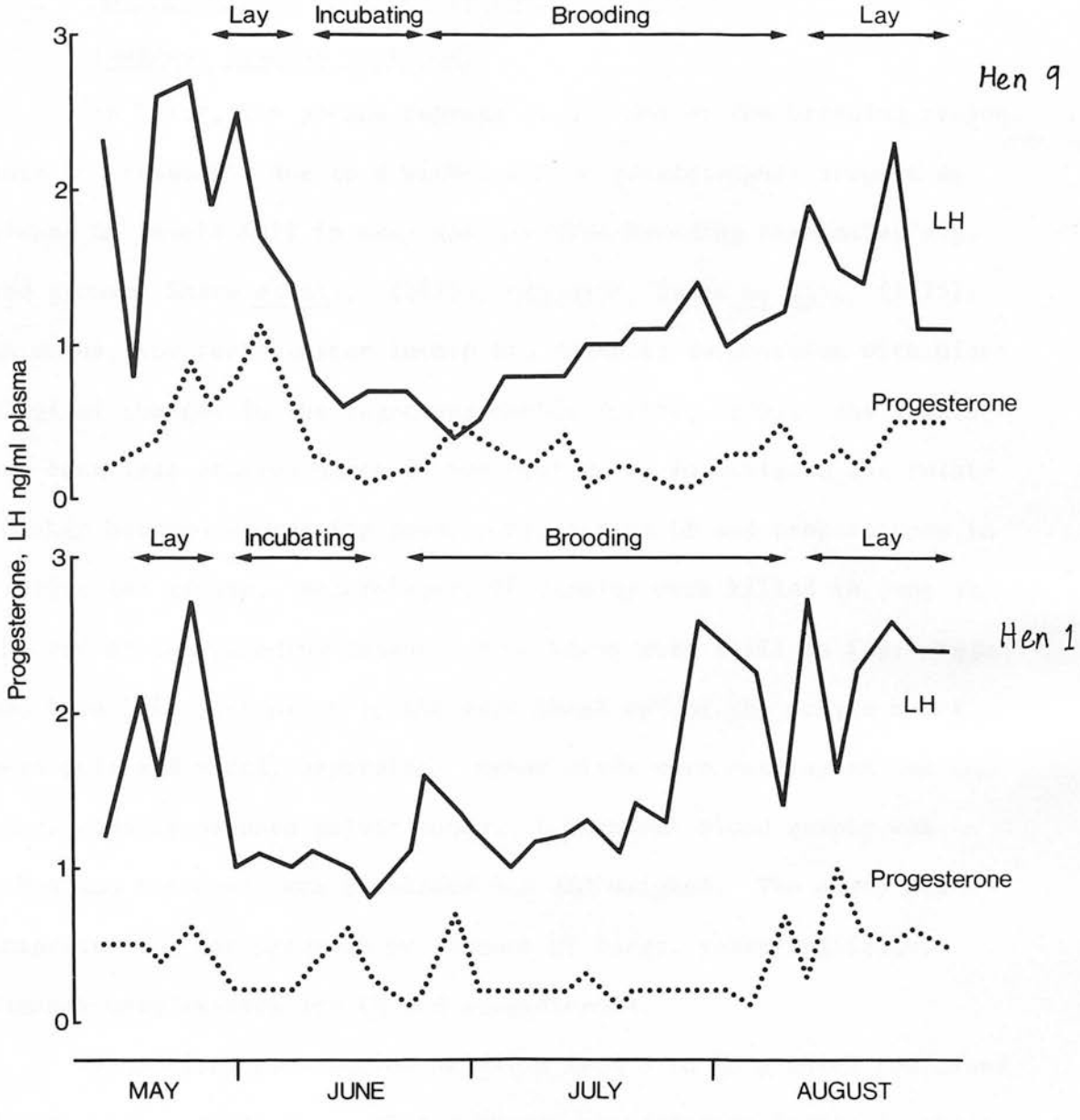
B = Brooding

? = Possibly laying out of nest box

R = Chicks removed

Figure No. 2.

THE CONCENTRATIONS OF LUTEINIZING HORMONE AND PROGESTERONE THROUGHOUT THE BREEDING CYCLE IN BANTAM HENS .



birds. The variable length of the laying and brooding periods made it difficult to mean the data for all nine hens. However, two examples of the hormone profiles throughout a cycle of broodiness are shown in Figure 2, and full data are in Tables 4 & 5.

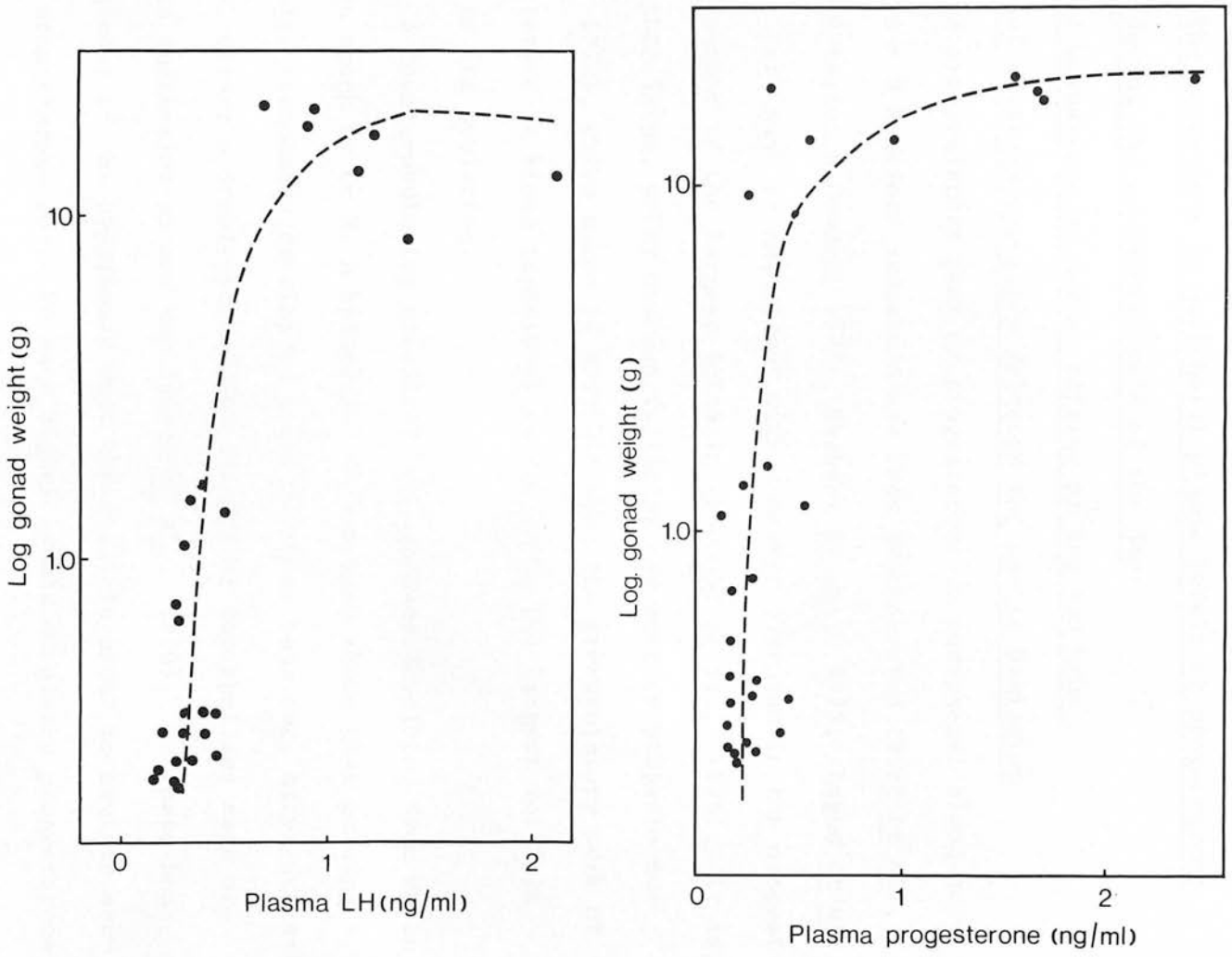
4.2 The Relationship between Blood Levels of Progesterone, LH and Ovarian Activity in the Red Grouse (Lagopus lagopus scoticus)

In birds, the gonads regress at the end of the breeding season. This is presumably due to a withdrawal of gonadotrophic support as plasma LH levels fall in many species when breeding terminates e.g. red grouse, Sharp et al., (1975), mallards, Haase et al., (1975). In males, low testosterone levels are found in association with histological changes in the regressed testis (Lofts, 1975). The female has been less studied hence it was decided to investigate the relationship between decreasing gonad size, plasma LH and progesterone in captive red grouse. Accordingly, 28 females were killed in June at the end of the breeding season. Some birds were still in lay. Eggs had been laid just prior to the experiment and/or the pelvic bones were soft and widely separated. Other birds were not laying and had hard, closely apposed pelvic bones. A terminal blood sample was taken and the ovary was dissected out and weighed. The ovary was inspected for the presence or absence of large, yolky follicles. Plasmas were assayed for LH and progesterone.

Nine birds had ovaries weighing from 8 to 20 g which contained large, yolky, follicles. Plasma LH and progesterone levels in these birds were between 0.8 - 2.1 ng/ml (LH) and 0.2 - 2.5 ng/ml (progesterone) (Fig 3). If the ovary contained an atretic follicle, the hormone levels were at the lower end of the quoted ranges.

Figure No. 3

THE RELATIONSHIPS BETWEEN BLOOD LEVELS OF PROGESTERONE, LUTEINIZING HORMONES AND OVARIAN WEIGHT IN RED GROUSE



In the remaining 19 birds, the ovaries were either completely regressed, or nearly so. They weighed much less, between 1.8 - 0.02 g, and the plasma LH and progesterone levels ranged between 0.2 - 0.6 ng/ml and 0.1 - 0.55 ng/ml respectively. (Fig 3).

4.3 The variations in peripheral plasma levels of progesterone during the ovulatory cycle of the hen: A comparison of normal strains of hen and hens of a strain specially selected for double ovulation

A pre-ovulatory peak of progesterone in peripheral blood occurring 6 - 8 h before ovulation has been demonstrated (Furr et al., 1973; Peterson & Common, 1972; Shahabi et al., 1975; Lague et al., 1975). This peak is coincident with a marked increase in the progesterone content of the largest follicle (Shahabi et al., 1975). It is known that large, yolky ovarian follicles can secrete progesterone (Furr, 1973), which makes it possible that the pre-ovulatory peak of progesterone in blood represents secretion by the largest follicle prior to its ovulation.

A double-ovulating strain of hen has been developed from White Leghorn stock by Dr H. A bplanalp. It has been shown that growing follicles frequently develop in pairs in these hens and, after ovulation occurs, either a double-yolked egg results or two shelless eggs are laid in succession on one day (Sharp et al., 1976). In these hens, a consequence of the hypothesis that the follicle about to ovulate secretes progesterone would be much higher levels of plasma progesterone in the hours preceding ovulation than in normal hens. This was investigated by measuring progesterone levels in blood taken from double-ovulating hens over the period 2 - 18 h prior to an ovulation. To facilitate accurate comparison of progesterone plasma levels in double-ovulating and normal hens, the experiment was also performed using a mixture of Ross I and Babcock hens.

Table 6

Blood Progesterone Levels During The Ovulatory Cycle of Double Ovulating and Normal Hens.

i Double Ovulating Hens.

	Hours Before Ovulation									
	18	16	14	12	10	8	6	4	2	0
7D			2.1	2.5	2.2	1.9	5.5	2.0	7.1	
14D		1.8	1.8	2.0	2.3	7.4	8.2	6.1		
10D		3.2	3.5	3.2	4.2	6.5	10.9	10.0		
19D	3.2	3.1	2.2	2.4	3.1	8.1	8.6			
17D		3.0	3.2	2.7	3.4	7.7	9.1	7.9		
8D				3.1	8.1	13.8	15.0	9.7	3.8	2.9
16D	2.5	2.7	2.5	2.8	2.8	5.5	6.0			
		2.5	2.5	3.1	3.2	5.5	7.0	5.9		
MEAN	2.85	2.72	2.54	2.73	3.66[a]	7.05	8.79[b]	6.93	5.45	
S.E.M.	0.35	0.21	0.23	0.15	0.67	1.19	1.08	1.21	1.65	
n	2	6	7	8	8	8	8	6	2	

ii Normal Hens.

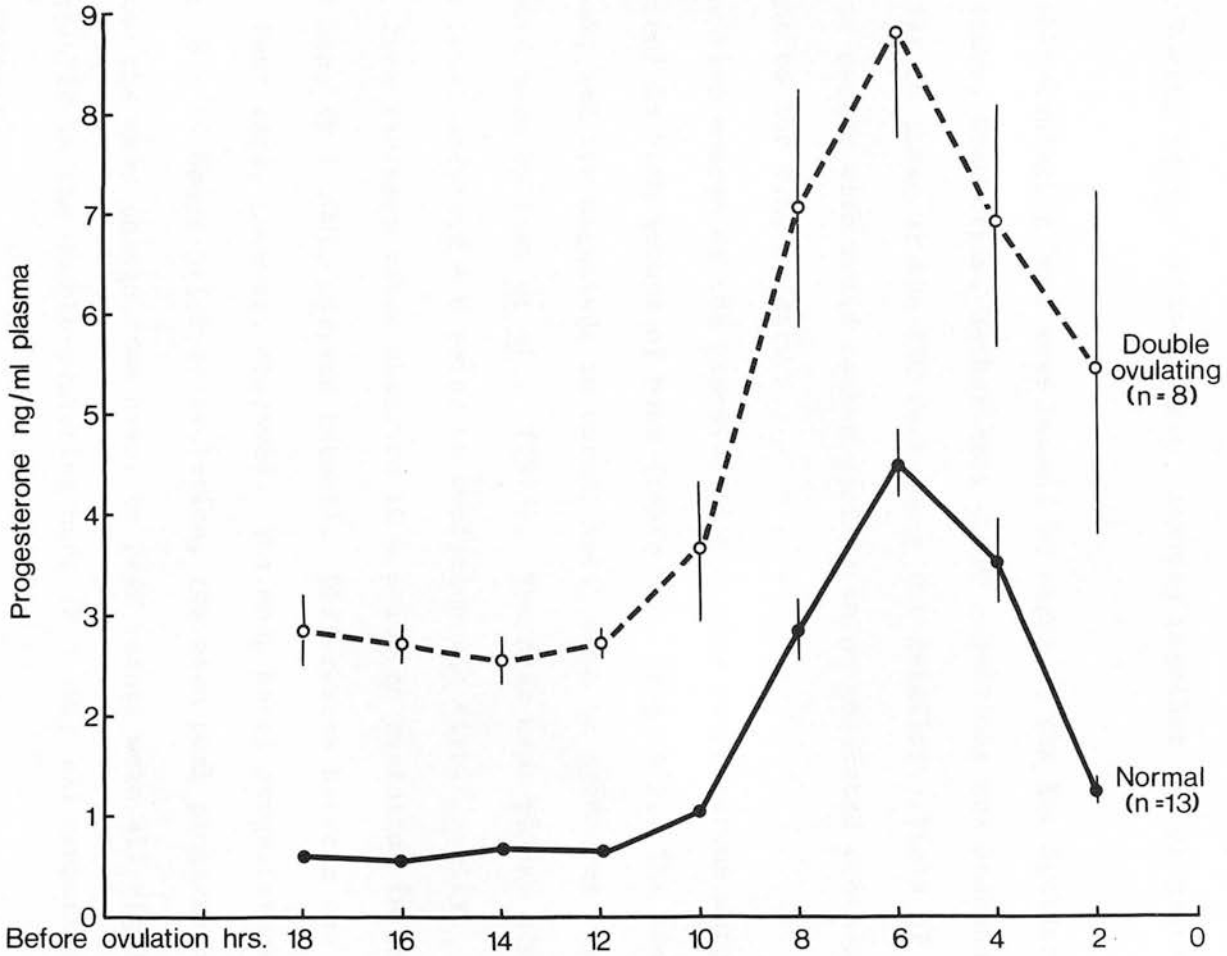
1		0.4	0.9	0.7	1.5	3.8	4.9	3.7		
2				0.3	1.1	3.4	6.4	6.2	1.37	0.45
3			0.8	1.2	1.2	2.4	3.4	2.6	1.7	
4			0.3	0.4	0.4	1.1	2.3	2.6		
5	0.6	0.6	0.6	0.5	1.2	2.2	5.1			
7			0.7	0.5	0.7	0.8	3.5	3.4	1.4	
8	0.7	0.7	0.6	0.7	1.13	3.7	5.3			
10	0.5	0.5	0.6	0.7	0.8	4.2	5.7			
11				0.8	0.5	3.1	5.8	5.0	1.2	0.6
14			0.9	0.7	2.4	3.1	3.2	1.3	0.6	
15				0.4	0.8	1.3	3.5	2.9	1.3	0.4
16				0.9	1.2	3.8	4.1	3.3	0.7	0.3
17				0.7	0.7	3.8	5.2	4.3	1.7	2.0
MEAN	.6	.55	.68	.65	1.05[a]	2.82	4.49[b]	3.53	1.25	0.75
S.E.M.	.06	.06	.07	.07	0.14	0.32	0.34	0.44	0.14	0.32
n	3	4	8	13	13	13	13	10	8	5

n = Number of observations

Means followed by different superscripts differ significantly ($P < .001$; paired t-test).

Figure No. 4

PROGESTERONE PERIPHERAL PLASMA LEVELS DURING THE OVULATORY CYCLE OF HENS SELECTED FOR DOUBLE OVULATIONS AND NORMAL HENS. (Means ± S.E.M.)



Seven 2.5 ml blood samples were taken from 8 double-ovulating and 17 normal hens starting at 18.00 h and continuing through the night to 6.00 h the following morning. Times of oviposition, and number and type of eggs laid by double-ovulating hens, were noted. Ovulations were calculated to occur within 14 - 45 min of oviposition (Warren & Scott, 1935). Plasmas were assayed together for progesterone.

Double-ovulating hens were housed in cages at the Het Spelderholt Institute, Beekbergen, Netherlands where conditions and husbandry were similar to those at the PRC (see above for details). Vials of plasma were packed with solid carbon dioxide in an insulated container and brought to the U.K. by air.

The time course of the pre-ovulation plasma progesterone surge was identical in both groups of hens (Table 6), (Fig 4). The timing of the peak, and its magnitude in normal hens, were in agreement with measurements made by Furr et al., (1973). The peak mean plasma progesterone level observed 6 h prior to ovulation was significantly different from the mean value observed 10 h prior to ovulation in both groups of hens ($P < .001$; paired t-test). Differences between the two groups of hens were, however, observed. The mean basal progesterone levels at 18 - 14 hours prior to ovulation, the mean peak progesterone levels, and the mean change from basal to peak values were all significantly greater in the double-ovulating hens ($P < .01$; all comparisons; unpaired t-tests).

4.4 Control of the pre-ovulating luteinizing hormone surge in the chicken : the role of progesterone and androgens

The pre-ovulatory release of LH in the hen is associated with increased concentrations of plasma oestrogens, progesterone and testosterone (p. 18).

The role of these steroids has been investigated in intact and ovariectomised hens. Experimental evidence shows that injection of progesterone and testosterone, but not of oestrogens, stimulates LH secretion in the laying hen (Wilson & Sharp, 1975b, 1976a). In the oestrogen / progesterone primed long-term ovariectomised hen progesterone, but not testosterone or oestradiol, induces LH release (Wilson & Sharp 1976b). These observations suggest that increased secretion of progesterone and/or testosterone by the largest ovarian follicle as it approaches ovulation triggers the pre-ovulatory release of LH. This has been investigated further by comparing changes in the concentrations of progesterone and androgens in the same peripheral blood samples with respect to:

a. The maturity of the largest follicle.

b. The initiation of the pre-ovulatory LH surge.

4.4.1 Changes in the concentration of progesterone and androgens in peripheral blood in relation to the maturity of the largest ovarian follicle

Blood samples were taken at hourly intervals for 3 h starting at 16.00 h on a day when the terminal egg (E_t) of a sequence was laid between 14.00 and 15.00 h. Blood samples were therefore taken between 26 and 30 h after the terminal ovulation (O_t) of a sequence. At this time the largest ovarian follicle would have been in an ovulable condition and be due to ovulate early in the morning of the following day (Fraps, 1955).

Two days later, blood samples were again taken at hourly intervals for 3 h starting at 13.00 h following the first oviposition which occurred between 07.30 and 08.30 h. The first oviposition of a sequence was taken to occur at the same time as the second ovulation (O_2) of a sequence (Fraps, 1955). The blood samples were there-

fore taken between 5 h and 9 h after an O_2 ovulation. At this time the largest ovarian follicle would not have achieved an ovulable condition (Fraps, 1955). Concentrations of plasma progesterone and androgens could thus be measured in the same hen when the largest ovarian follicle was mature or immature. This experimental approach takes advantage of the fact that following an O_t ovulation the largest ovarian follicle becomes mature but cannot ovulate until after the start of the 'open period' of the ovulation cycle which begins at the onset of darkness (Fraps, 1955).

Mean concentrations of progesterone and androgens were about 30% higher when the ovary contained a mature follicle (26 - 30 h after an O_t ovulation) than when the largest ovarian follicle was immature (5 - 9 h after an O_2 ovulation) (Table 7). This difference was significant for progesterone ($P < 0.05$) but not for androgens ($P < 0.1$).

4.4.2 Changes in the concentration of progesterone and androgens in peripheral blood at the time of initiation of the pre-ovulatory LH surge

Blood samples were taken at 30 minute intervals between 1600 h and midnight in hens which were predicted to have an O_1 ovulation early in the morning of the following day. In such birds, pre-ovulatory LH levels start to rise soon after the onset of darkness (Wilson & Sharp, 1973). Plasmas were analysed for concentrations of LH, progesterone and androgens.

Plasma androgens were measured using the RIA described by Sanwal, Sundby & Edqvist, (1974) without chromatographic purification. The antiserum showed an important cross-reaction with 5α - dihydrotestosterone and an estimate was made of the concentration of this steroid to the total androgens measured. Plasma samples from laying

Table 7

Progesterone and androgen concentrations in the peripheral blood of the laying hen in relation to the maturity of the largest ovarian follicle

mean ± S.E.M.

(a) Ovary does not contain a mature follicle (5-9h after C_2 ovulation)

Time	No. of hens [+]	Progesterone (ng/ml)	Androgens (ng/ml)
13.00	7	1.05 ± 0.15	0.43 ± 0.08
14.00	7	0.99 ± 0.12	0.33 ± 0.03
15.00	7	0.93 ± 0.11	0.30 ± 0.02
16.00	7	0.81 ± 0.09	0.44 ± 0.08
Total mean		0.95 ± 0.06	0.37 ± 0.04

(b) Ovary contains a mature follicle (26-30h after C_t ovulation)

Time	No. of hens [+]	Progesterone	Androgens
16.00	7	1.27 ± 0.18	0.41 ± 0.03
17.00	7	1.26 ± 0.11	0.45 ± 0.05
18.00	7	1.10 ± 0.14	0.51 ± 0.08
19.00	7	1.30 ± 0.16	0.47 ± 0.02
Total mean		1.24 ± 0.07*	0.46 ± 0.02[§]

[+] The same seven hens were used for (a) and (b).

* $P<0.05$ (paired t test) compared with mean for birds studied 5-9h after C_2 ovulation.

§ $P<0.1$ (paired t test) compared with mean for birds studied 5-9h after C_2 ovulation.

hens were chromatographed in sephadex LH-20 columns following the method of Auletta, Caldwell & Hamilton, (1974) to separate 5α - dihydrotestosterone from testosterone. The apparent androgen concentrations in the 5α - dihydrotestosterone fraction was 0.15 ± 0.04 ng/ml (mean ± S.D., n = 5) and in the testosterone fraction, 0.44 ± 0.16 ng/ml (n = 5). Plasma aliquots were assayed in duplicate after extraction with light petroleum. Intra- and inter-assay co-efficients of variation were 3.9% and 9.8% (n = 10) respectively.

Students t-test was used for all statistical calculations.

The pre-ovulatory increase in plasma LH levels was observed to be biphasic, with a moderate rise around 10.5 h before ovulation followed about 2 h later by a much steeper increase. (Figs. 5 & 6). Although this biphasic increase was seen when the data were pooled (Fig. 5), it was better illustrated by individual examples (Fig. 6). The first increase in LH concentration occurred immediately after the onset of darkness and usually took the form of a small 'blip'; the second increase occurred much more abruptly than was suggested by the pooled data (cf. Figs 5 & 6).

The concentration of plasma progesterone fell initially, around the onset of darkness, and then increased in two stages (Figs 5 & 6). The pooled data suggested that the level of progesterone was falling well before the onset of darkness (Fig 5) but a careful comparison of progesterone and LH concentrations in individuals (Fig 6) showed that the dip in the progesterone level was only slightly in advance of the LH 'blip'. Following this dip, the concentration of progesterone increased steadily and although not shown in the pooled data (Fig 5) continued to do so until it was 30 - 40% above the level observed immediately before the onset of darkness (Fig 5). At this point, a second and much steeper increase occurred which coincided precisely

with the secondary steep increase in the concentrations of plasma LH.

Plasma androgen concentrations generally followed the same pattern as the LH levels (Figs 5 & 6). There was a small 'blip' immediately after the onset of darkness followed by a sustained steep rise coinciding with the secondary increase in progesterone levels at about 8.5 h before ovulation. Relatively high androgen concentrations were found in some birds at the beginning of the sampling period before the onset of darkness (Fig 6).

Fig 5

The concentrations of LH (●———●), Progesterone (o------o), and Androgens (▪———▪), in the plasma of twelve laying hens before and during the initiation of the pre-ovulatory hormone surges (Means ± SEM).

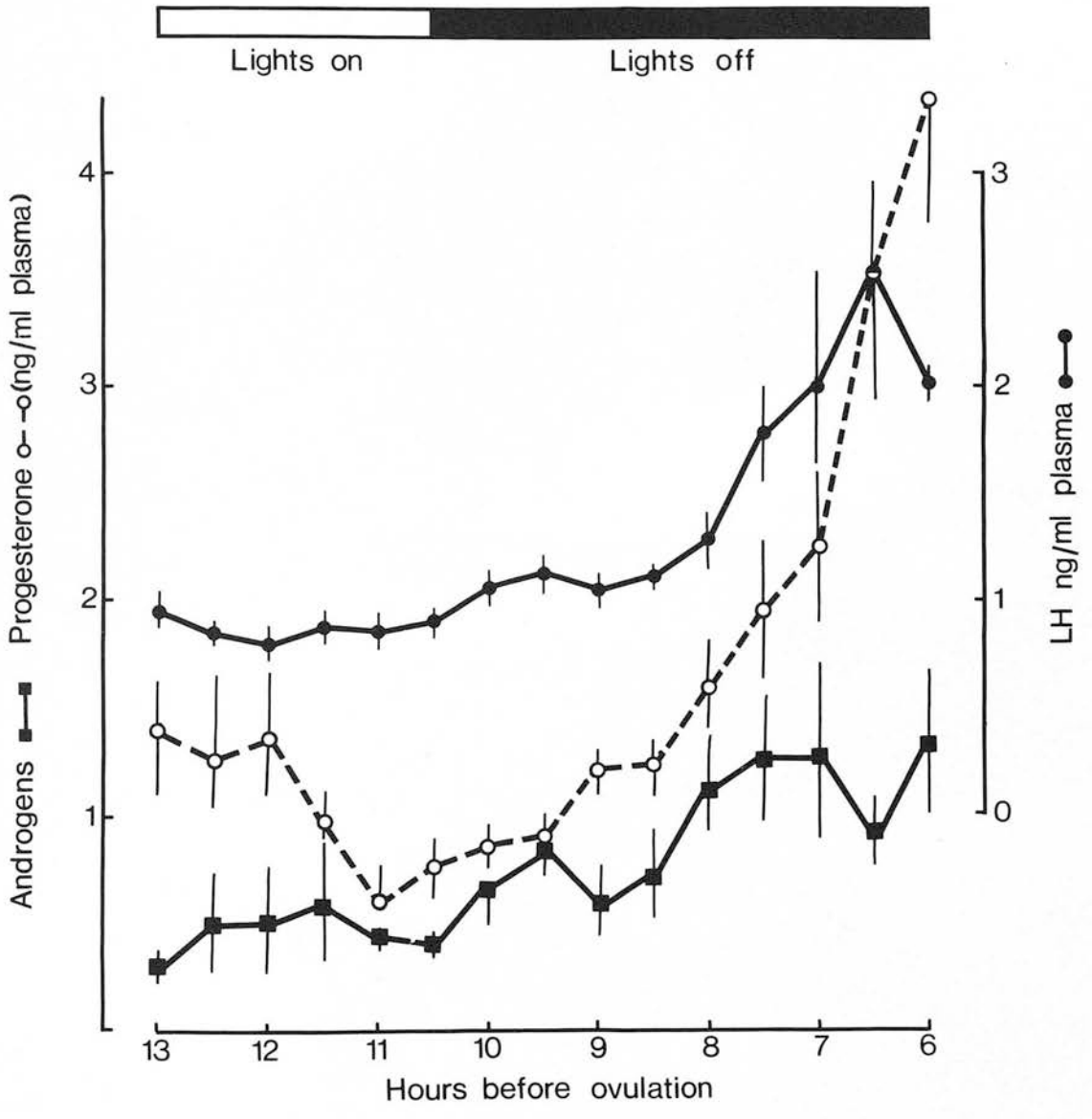
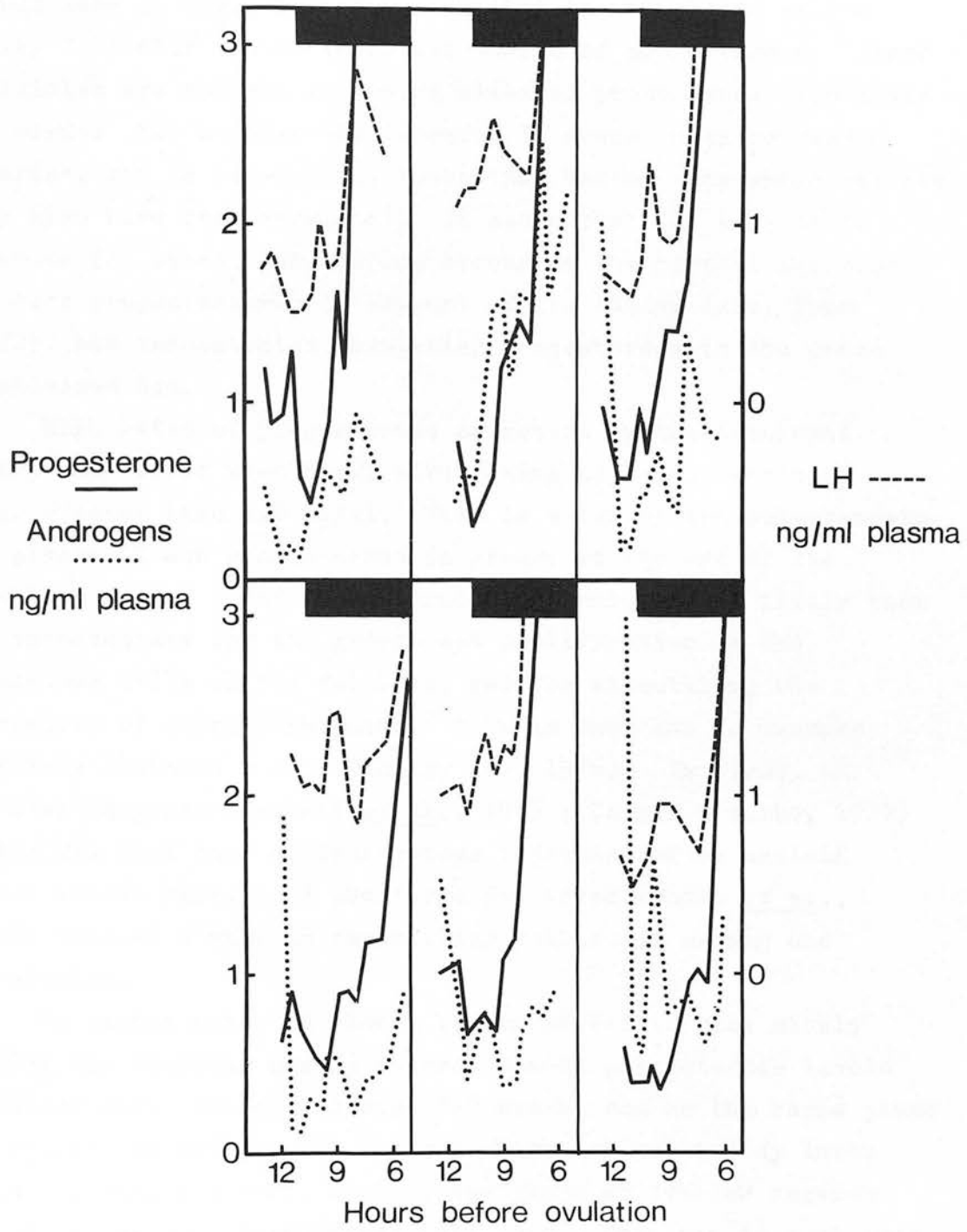

Lights on
Lights off
Androgens ■—■ Progesterone o– –o(ng/ml plasma)
LH ng/ml plasma ●—●
4
3
2
1
3
2
1
0
13
12
11
10
9
8
7
6
Hours before ovulation

Fig 6

Six samples of the concentrations of LH (------), Progesterone (————), and Androgens (.....) in the plasma of laying hens before and during the initiation of the pre-ovulatory hormone surges.


Progesterone
Androgens
ng/ml plasma
LH -----
ng/ml plasma
3
2
1
0
12 9 6 12 9 6 12 9 6
Hours before ovulation

Discussion

In the experiments performed on grouse and bantam hens, basal plasma progesterone levels tended to be higher when the birds were in lay. This suggests that the developed yellow yolky follicles are an important source of progesterone. These follicles are not the exclusive sites of progesterone synthesis in birds. The hormone was detected in grouse with regressed ovaries, and in non-laying, incubating bantam hens whose ovaries may also have been regressed. It seems that the very small ovarian follicles, the ovarian stroma or the adrenal may also produce progesterone. In support of the latter idea, Furr (1973) has demonstrated circulating progesterone in the ovariectomized hen.

High rates of progesterone secretion by the developed ovary only occur when basal circulating LH levels are high i.e. greater than 1.0 ng/ml. This is shown by the measurements of plasma LH and progesterone in grouse at the end of the breeding season , and in the broody bantams. It is likely that LH is necessary for the growth and proliferation of the granulosa cells of the follicle, and for stimulating the secretion of steroid hormones. This is the case in mammals (review: Richards & Rees Midgley Jr., 1976). In birds, LH is steroidogenic (Shahabi et al., 1975 ; Camper & Burke, 1977) while the fact that an intravenous injection of an anti-LH serum causes atresia of the large follicles (Sharp et al., 1978) implies a role in controlling follicular growth and maturation.

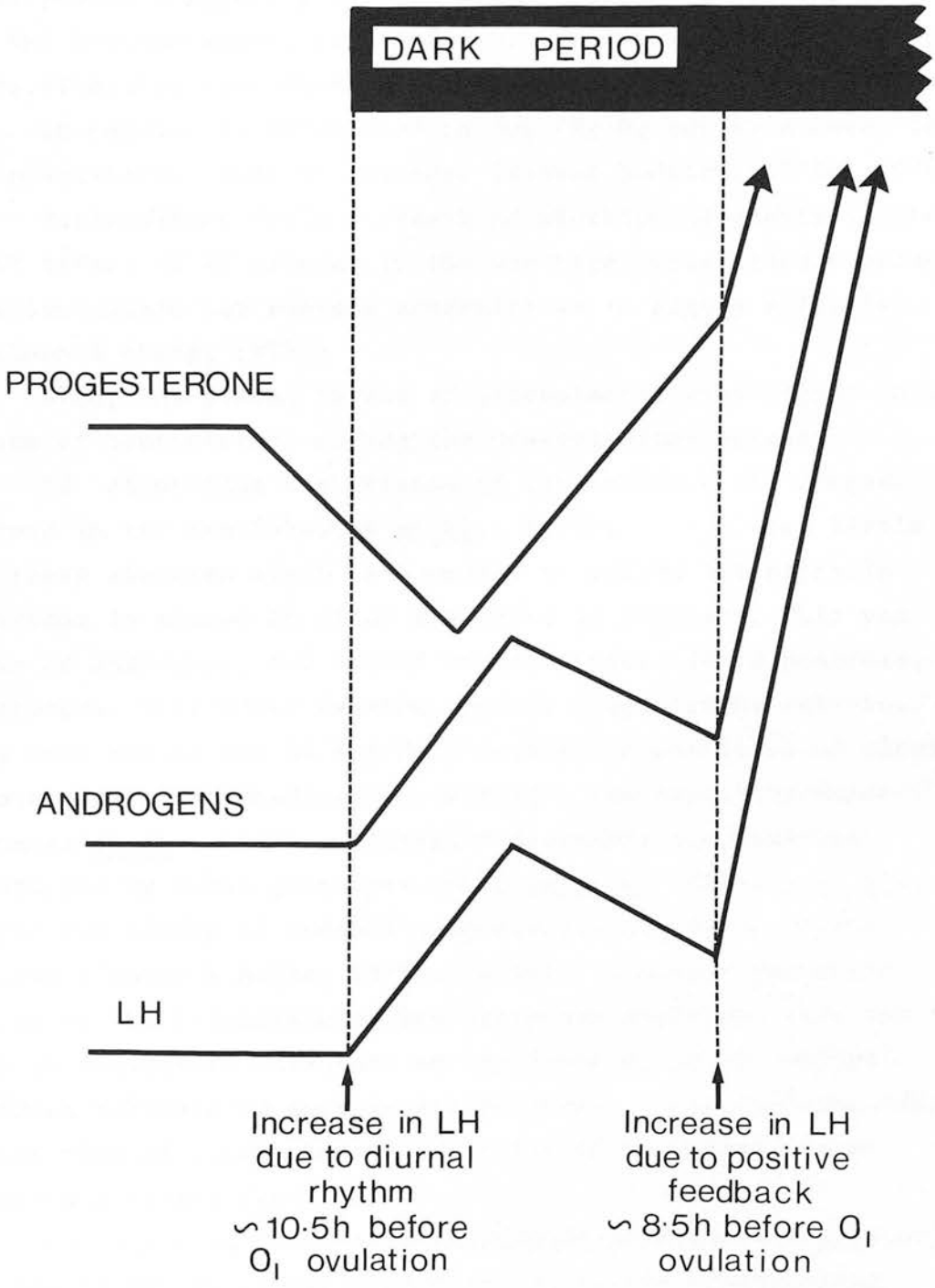
In bantam hens, LH plasma levels tended to rise slowly during the brooding period whereas plasma progesterone levels remained low. Brooding lasted 5-7 weeks, and as the rapid phase of follicular development in hens which are not broody lasts about 11 days (Gilbert, 1971a), the phase of ovarian regeneration in brooding birds seems prolonged. This may be explained by the plasma LH levels in the brooding period, which, although they were higher than in the incubating period, were lower than the levels recorded for laying hens (Table 4). The amount of LH stimulation may only have been sufficient for a slow regen-

eration of ovarian follicles. Possibly, the pres ence of chicks supressed LH secretion via a direct neural inhibitory effect. Removal of the chicks (hens 2,6, & 7 ; Table 4) resulted in a rapid increase in plasma LH levels and resumption of lay.

A more precise indication of the site of progesterone secretion in the active ovary may be deduced from the data on double-ovulating hens. These hens exhibited pre-ovulatory peaks and basal levels of progesterone that were approximately twice as high as peaks or basal levels recorded in single-ovulating hens. This makes it likely that the follicle(s) next due to ovulate secrete(s) progesterone at this stage of the ovulatory cycle. This possibility has been suggested by Shahabi et al., (1975a) who noted that the progesterone content of the largest follicle increased greatly in synchrony with the increase of plasma progesterone prior to ovulation. A further possiblity is that high basal and cyclic secretion of LH resulted in high plasma progesterone levels in double-ovulating hens. However, Sharp et al.(1976) have reported similar basal and pre-ovulatory LH levels in double-ovulating and single ovulating hens. Further evidence to support the idea of progesterone secretion by the largest ovarian follicle was obtained by the comparison of basal plasma progesterone levels which were higher when the largest ovarian follicle was mature than when it was immature.

The determinations of progesterone, androgens and LH concentrations in the same plasma samples taken at 30 minute intervals in the period preceding ovulation enable a conclusion to be drawn regarding the role of progesterone in the induction of the pre-ovulatory release of LH. This interpretation of the plasma hormone changes is shown in Figure 7 . The initial increase in plasma LH just after the onset of darkness is due to an underlying diurnal rhythm. It occurs in laying hens on nights when there are no pre-ovulatory LH surges (Wilson & Sharp, 1973) and is not due to the positive feedback action of gonadal steroids because it occurs in pre-pubertal pullets (Scanes et al., 1978) in which the positive feedback mechanism is refractory (Wilson & Sharp, 1975c). The secondary and

Figure 7: Diagram illustrating the role of progesterone in the control of pre-ovulatory release of LH.


DARK PERIOD
PROGESTERONE
ANDROGENS
LH
Increase in LH due to diurnal rhythm ~10·5h before O_1 ovulation
Increase in LH due to positive feedback ~8·5h before O_1 ovulation

steeper increase in plasma LH at around 8.5h before ovulation thus corresponded to the start of the pre-ovulatory surge. Since this was preceded by an increase in the concentrations of both progesterone and androgens, these steroids could be involved in triggering LH release by positive feedback. However, at the hypothalamo-pituitary level, progesterone is likely to be more effective than androgens for the following reasons:

1) LH release is stimulated in the laying hen by a lower dose of progesterone than of androgen (Wilson & Sharp, 1975b, 1976a)

2) Testosterone fails to exert an unambiguous positive feedback effect on LH release in the oestrogen/progesterone primed ovariectomized hen whereas progesterone is highly effective (Wilson & Sharp, 1976b)

Also, the plasma levels of progesterone were higher than those of testosterone during the pre-ovulatory surges.

LH stimulates the release of testosterone and progesterone in the hen (Shahabi et al., 1975). The plasma levels of these steroids might be expected to follow the biphasic increase in plasma LH after the onset of darkness. This was true of androgens, but not of progesterone. It is possible, therefore, that other factors control progesterone release. One such factor may be the fall in the concentration of plasma prolactin which immediately precedes a pre-ovulatory surge (Scanes et al., 1977). Prolactin depresses progesterone synthesis by human granulosa cells in vitro (McNatty et al., 1974) and blocks LH induced progesterone secretion in the turkey (Camper & Burke, 1977). A fall in plasma prolactin prior to the initiation of the steep pre-ovulatory rise may remove an inhibitory influence on the capacity of the mature ovarian follicle to secrete progesterone. Thus, the secondary steep rise of plasma LH may be initiated by progesterone-induced positive feedback.

Androgens may also have influenced progesterone production by the mature follicle. Androgens stimulate progesterone production by pre-antral rat granulosa cells in vitro (Lucky et al., 1977) and augment FSH induced progesterone secretion of cultured rat granulosa cells (Nimrod & Lindner, 1976 ; Armstrong & Dorrington, 1976). The rise in androgens just

after the onset of darkness may have stimulated progesterone production prior to the initiation of the pre-ovulatory rise in plasma LH. This view is supported by the observation that an injection of testosterone will reliably stimulate LH release only when the ovary contains a mature follicle (Wilson & Sharp, 1976a).

If there is a diurnal rhythm of sensitivity in the positive feedback mechanism governing LH release with increased sensitivity in the 'open period' as predicted by Fraps (1955), this study suggests that LH release should be triggered by plasma concentrations of progesterone in the order of 1.0-1.7 ng/ml during the open period (which starts at the onset of darkness) but not during the 'closed period' of the ovulatory cycle. Attempts to show that the sensitivity of the positive feedback mechanism during the 'open period' of the cycle is greater than during the 'closed period' have failed because the amounts of progesterone injected would have resulted in plasma concentrations far in excess of 1.0-1.7 ng/ml (Wilson & Sharp, 1975b ; Etches & Cunningham, 1977).

The observations of plasma progesterone and androgen levels corresponding to different stages of ovarian follicular maturity are consistent with the suggestion by Fraps (1955) that the concentration of 'excitation hormone' increases as the largest ovarian follicle matures. However, there is no convincing evidence for or against Fraps' view that the timing of the 'open period' of the ovulatory cycle is due to a change in the sensitivity of the positive feedback mechanism governing LH release. These data suggest an alternative mechanism which controls the timing of the open period. In this model, it is unnecessary to propose that the follicle secretes more progesterone as it matures, rather it acquires the capacity to sustain a prolonged release of progesterone in response to a small increase in the base-line concentration of plasma LH. This hypothesis is supported by the observation that sub-cutaneous administration of LH-RH is far more effective at promoting a prolonged release of progesterone when the largest ovarian follicle was mature than when it was immature (Etches & Cunningham, 1977).

4.5 The Effect of Age on the Times of Oviposition in a Flock of White Leghorn Type Hens

This experiment was performed with two aims in mind. The first was to determine whether the times of lay of the first and last eggs of a sequence of any given length stay constant throughout the first year of lay. Secondly, it was of interest to determine whether the patterns of lag within sequences in a modern strain of hen differ from those described for older strains by Fraps (1955) and Heywang (1938).

Paper tape records of egg-laying times for 62 PRC White Leghorn hens (derived from Shaver stock) were collected for 12 months starting in the second month of lay and analyzed by computer program. The egg production percentages for each month were calculated, also the mean lag and cumulative lag (cumulative lag = sum of lags between all eggs of a sequence) were calculated for all recorded sequences. The times of lay of the first and last eggs of a sequence were calculated for second and third months of lay and compared with the times of lay for the tenth and eleventh months. This was done for sequences of one to seven eggs.

Egg production throughout the year tended to decline, although this decline was erratic (Fig. 8). The patterns of lag for sequences, irrespective of the time of year when they were laid are shown in figure 9 along with patterns of lag taken from Heywang's (1938) data. Sequence lengths for which less than four examples were available were excluded. A noticeable feature of both sets of data is the increased lag at the beginning and the end of a sequence. The mean lag between successive eggs of a sequence, and the total lag between the first and last eggs of a sequence, for sequence lengths of 2-6 eggs are shown in Table 8 together with

the data presented by Fraps (1955). Total lag and mean lag do not differ significantly between the two sets of data for sequence lengths of 4 or more eggs. For 2 and 3 egg sequences, total lag and mean lag are less in the hens studied in this experiment than in Frap's (1955) report.

The times of lay of the first and last eggs of a sequence do not differ significantly between months 2-3 and months 10-11 of production (Table 9). The total lag increases as the sequence length increases from 2 to 4 eggs to a maximum of about 7h 30 min. (Table 9).

Table 8

Comparison of mean lag between successive eggs of a sequence, and total lag between first and last eggs of a sequence (Hours & Minutes)

Sequence Length		Number of Sequences	Total Lag	Mean Lag
2	a	660	3.41	3.41
	b	694	4.31	4.31
3	a	620	5.54	2.56
	b	399	6.40	3.20
4	a	496	7.15	2.25
	b	105	7.22	2.27
5	a	335	7.41	1.55
	b	28	7.45	1.56
6	a	219	7.49	1.34
	b	12	8.25	1.37

a. Data from this experiment; all months pooled

b. Data from Fraps (1955)

Table 9

Times of Lay (Hours & Minutes) of First and Last Eggs of a Sequence, and Difference between these times, in months 2-3 and 10-11 of egg production of White Leghorn hens (Means + Standard Deviation)

		MONTHS 2-3 OF PRODUCTION				MONTHS 10-11 OF PRODUCTION			
		NO. OF SEQUENCES	TIME OF FIRST EGG	TIME OF LAST EGG	TOTAL LAG	NO. OF SEQUENCES	TIME OF FIRST EGG	TIME OF LAST EGG	TOTAL LAG
SEQUENCE LENGTH	1	75	11.18±3.37			191	11.3±4.08		
	2	119	9.00±2.02	12.53±2.08	3.54±1.32	117	8.28±2.54	11.15±2.53	2.47±3.42
	3	102	7.56±2.01	13.47±2.07	5.51±3.03	104	6.55±1.29	11.49±3.24	4.55±3.21
	4	71	7.07±1.03	14.14±1.54	7.07±1.54	64	6.50±1.34	13.53±2.00	7.03±2.35
	5	59	7.05±1.50	14.06±2.02	7.01±2.42	35	6.46±2.22	13.36±2.03	6.50±3.24
	6	39	6.55±1.20	14.44±2.18	7.49±2.27	22	5.57±1.19	14.41±1.25	8.43±2.09
	7	19	7.48±2.50	13.36±2.32	5.47±2.57	14	7.47±2.22	15.08±1.12	7.21±2.37

Figure 8

Egg production of White Leghorn type hens kept at King's Buildings. Dotted line indicates trend of decreasing production with age.

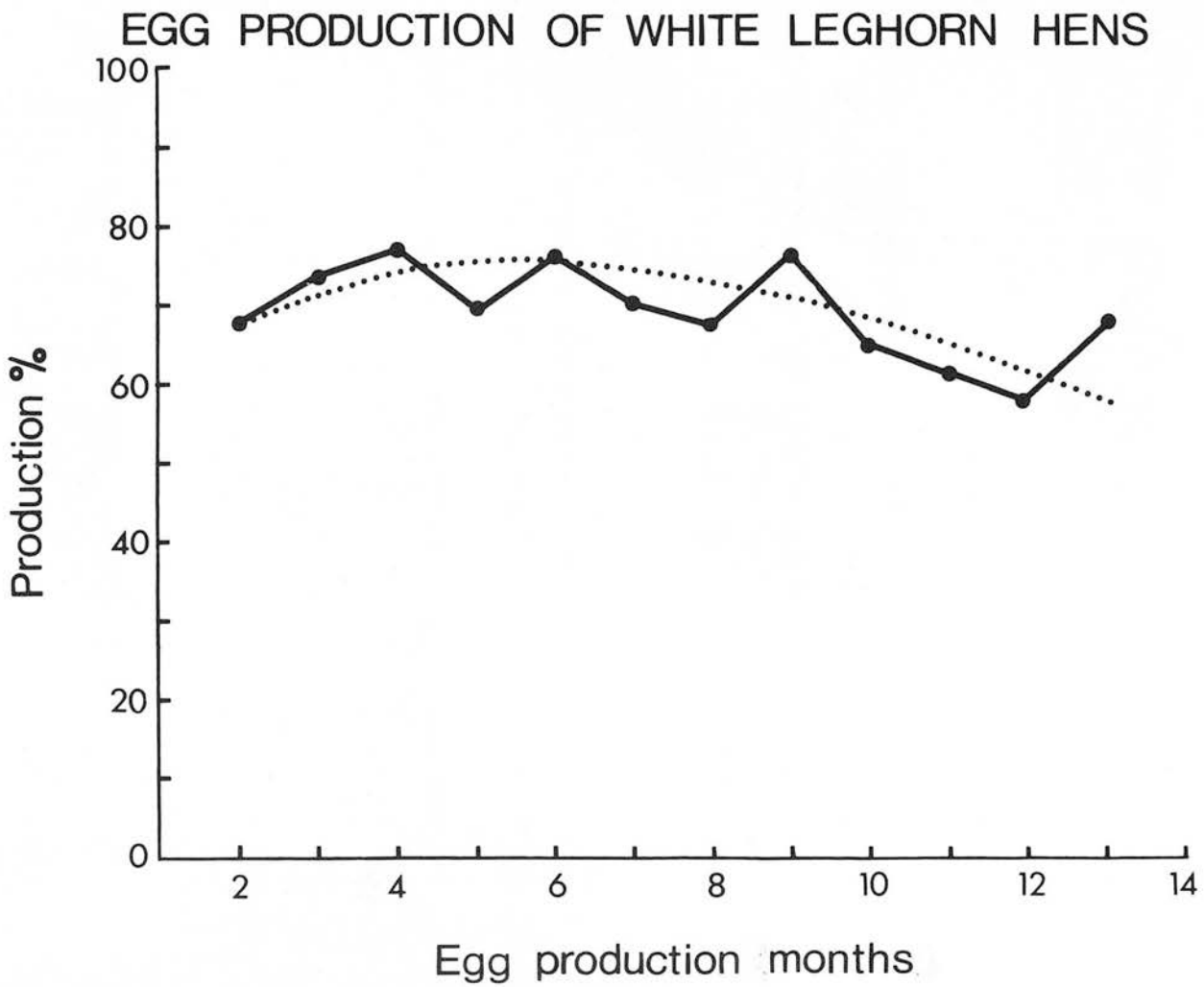
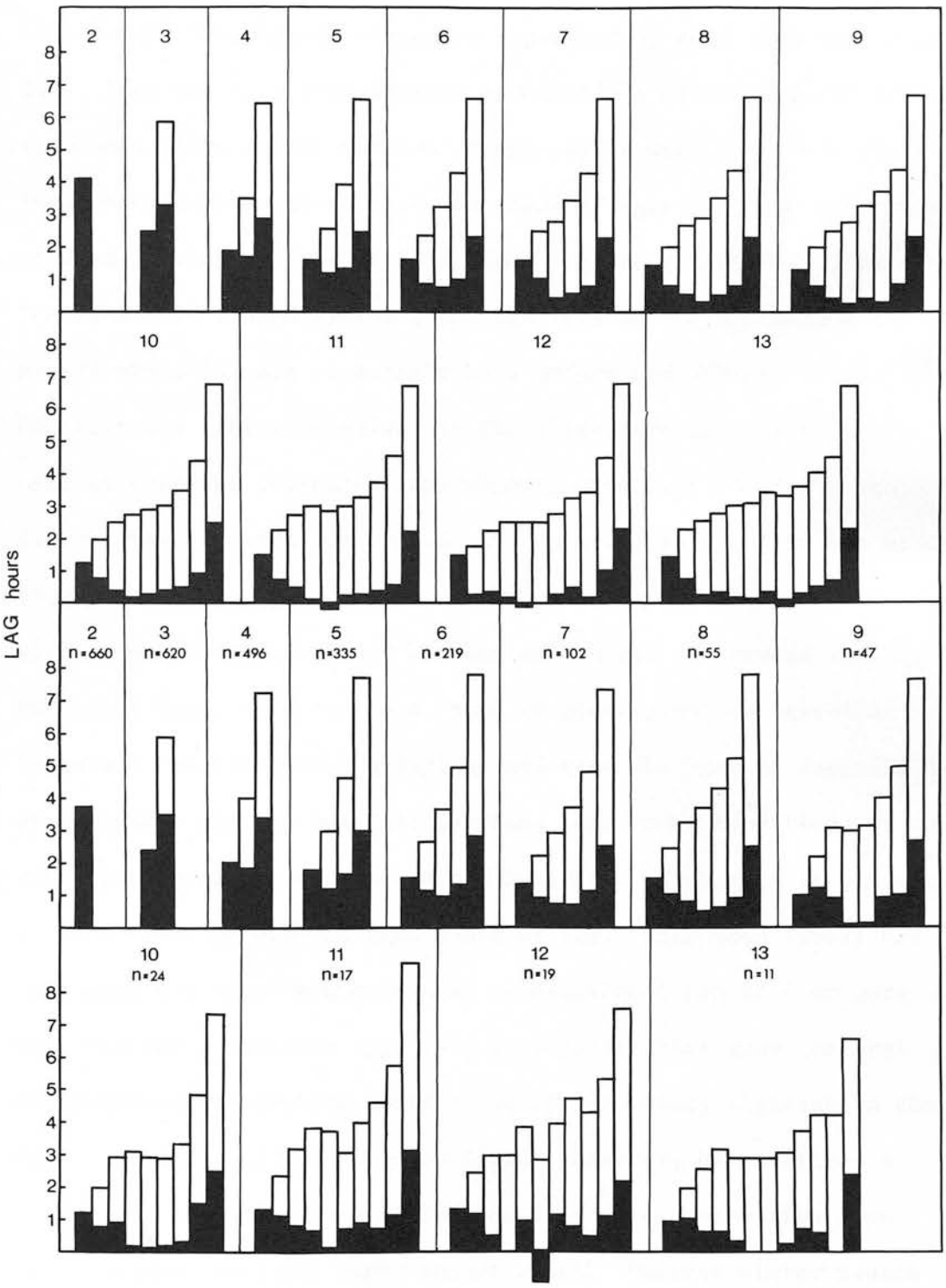

EGG PRODUCTION OF WHITE LEGHORN HENS
Production %
100
80
60
40
20
0
2
4
6
8
10
12
14
Egg production months

Figure 9

Diagrams of lag (shaded bars) and total lag (open bars) in a sequence. Figures above each diagram denote sequence length.

Top two rows - data from Heywang (1938)

Bottom two rows - data from White Leghorn hens housed at King's Buildings. 'n' indicates number of sequences used to compute lag and total lag times.


LAG hours
2
3
4
5
6
7
8
9
10
11
12
13
n=660
n=620
n=496
n=335
n=219
n=102
n=55
n=47
n=24
n=17
n=19
n=11

4.6 The Effect of Age on Laying Patterns in Three Strains of Hen

The decline in egg production as flocks grow older can be accounted for in several ways. Ageing hens may lay shorter sequences and/or more 'uncoupled' sequences separated by more than one pause day. They may also show various combinations of coupled and uncoupled sequences. The normal pattern of egg-laying where only one pause day intervenes between successive sequences of eggs has been extensively studied (review: Fraps, 1955). Such sequences have been termed 'coupled' or 'closed cycles', and the reasons for the single day of missed ovulation are reasonably well understood (Fraps, 1955). Considerable confusion exists in the literature as to the terminology used to describe sequences separated by more than one day. Fraps (1965) uses the term 'uncoupled' or 'open cycles', and in his study sequences were only considered to be uncoupled if five or more days with no egg laid intervened between sequences. Greenwood (1962), on the other hand, used the term 'gaps in production' to describe temporary cessation of egg-laying, and used the term to describe 4 or more days with no egg laid for hens kept under conditions of constant photoperiod, temperature and humidity. Such gaps in production did not occur before the 14th month of lay. Greenwood (1962) has also used the term 'winter pause' to describe a gap of 4 or more days between successive eggs laid by a pullet kept under natural environmental conditions modified by supplementary lighting in the winter months. Lerner & Taylor (1943), however, have defined a winter pause as cessation of lay for 7 or more consecutive days, and have also used the term 'annual rest'. Whereas winter pauses were apparently influenced by seasonal daylength and temperature, the annual rest was shown to be correlated with age. Lerner & Taylor (1943) calculated the mean duration of this rest as 85.5d,

and the mean age at the last egg before the rest as 80.3 weeks. Although these authors made no observations on moulting, they noted that the moult and the annual rest are closely associated phenomena. Greenwood (1962) reported that the mean gap in production associated with a moult was 32d for hens in a constant environment and used the term 'pause in production' rather than 'annual rest' to describe this period of lost egg production.

To the poultryman, such academic distinctions are of little interest: gaps, pauses, rests or uncoupled sequences represent 'lost' eggs and reduced profitability. It is important to know the extent to which periods of non-production contribute to the decline in first year production and to attempt to explain them in the light of current physiological knowledge of the endocrine control of egg-laying. A detailed analysis was therefore made of the patterns of egg-laying in three strains of hen (Ross I broiler breeder; Ross Ranger midweight egg-laying strain; Babcock B300 lightweight egg-laying strain) during the first 18 months after the onset of lay.

4.6.1

Survivors' Egg Production

The monthly totals of survivors are shown in Table 10 and the survivors' egg production graphs are shown in Fig. 11. The effect of age on egg production is clearly seen. The sharp fall in egg production in all strains between months 3-4 and the recovery from months 4-5 are of special interest; otherwise the trend of falling egg production corresponds with the description given above (p. 7).

4.6.2

Effect of Age on Sequence Length

The distribution of sequence length for months 2-3, 7-8, 12-13 and 17-18 were examined (Tables 12&13). It is evident that

one cause of the fall in egg production is the tendency for fewer long* sequences to be laid. For example, there are more single egg sequences per 100 birds in each consecutive period studied in all strains. Also, a progressively larger proportion of the Babcock and Ranger flocks lay single egg sequences as the hens age, although the proportion of Ross I hens laying single egg sequences declines as the birds age and production falls.

4.6.3

Analysis of Laying Patterns with Age

Laying patterns were defined in the following way:

1. Coupled sequences - sequences separated by only one day when no egg was laid.
2. Uncoupled sequences - sequences separated by 2-6 days when no egg was laid.
3. Rest - a period of 7 or more days when no egg was laid. Birds were only classified as resting in a particular laying month if they resumed egg-laying subsequently.

Using these definitions, the laying performances of flocks in months 3-20 of lay were classified into seven categories -

a. Coupled sequences only
b. Uncoupled sequences only
c. Rest only
d. Coupled and Uncoupled sequences
e. Coupled sequences and rest
f. Uncoupled sequences and rest
g. Coupled and uncoupled sequences and rest.

* 'long' sequence defined as > 4 (Ross I), > 5 (Ranger), > 6 (Babcock)

Complete data appear in Table 14.

In the case of Babcock and Ranger hens, a progressively smaller percentage of the flock laid coupled sequences as the birds aged. This trend was only evident from month 5 onwards since the percentage laying coupled sequences fell abruptly to zero from months 3-4 and then rose equally sharply between months 4-5. This fall was accompanied by an equally marked rise in the proportion of birds laying coupled and uncoupled sequences in month 4. The percentage of Ross I hens laying coupled sequences declined from months 3-5, rose during months 5-7 and declined steadily thereafter.

The numbers of birds laying uncoupled sequences only were either zero or negligible and no pattern was discernible.

The percentage of Babcock hens classified in category C (Rest only) fluctuated. The mean percentage was 5.3 $\pm$ 2.6 (standard deviation). More Ranger hens appeared in this category with increasing age and the number rose sharply between months 13-14 and remained relatively stable thereafter. Apart from a minor peak in month 4, few Ross I hens appeared in category C between months 3-9, but the percentage rose between months 9-14 and then fell to zero or insignificant levels.

Mention was made earlier of the high proportion of Babcock and Ranger hens observed in category D (coupled and uncoupled sequences) in month 4. This aside, there was a steady increase in the number of Babcock hens in this category throughout the period studied. In the case of the Rangers, apart from the peak in month 4 numbers rose until month 9 and remained fairly stable thereafter. Ross I hens showed a different pattern. Numbers were high initially and remained so until month 9 after which the percentage declined reaching and maintaining a new plateau from months 12-20.

The number of Babcock hens in category E (coupled sequences and rest) was always low: the mean percentage was 3.01 ± 2.11 (standard deviation). Likewise, the percentage of Ranger hens in this category was low: mean % 1.67 ± 1.28 (standard deviation). More Ross I hens showed this pattern of laying and the percentage ranged from 4.6 to 22.8. Numbers tended to be lower up to month 12, and in the upper range thereafter.

The numbers of hens laying uncoupled sequences and showing rests (category f) were low in all three strains. The percentage of hens fluctuated around means of: (Babcock) 1.84 ± 1.28 (standard deviation); (Ranger) 1.64 ± 1.58. Up to month 10, less than 3% of Ross hens appeared in this category, while between months 11-20, the percentage ranged from 3.7 - 9.4.

With respect to the final category 'g' (coupled sequences, uncoupled sequences and rest), only a small proportion (< 5%) of Babcock hens were classified in this category up to month 14, and from 6.3 - 13.5% thereafter. A similar pattern was observed in the flock of Ranger hens. Less than 10% (and less than 5% in several months) appeared in this category up to month 14, although the numbers rose thereafter. Rather more Ross I hens were classified in category g. Initially, the percentage was 10.5 and numbers followed an increasing trend reaching 46.9% in month 20.

The highest rates of lay were achieved by hens laying coupled sequences, followed by hens laying a combination of coupled and uncoupled sequences. Medium to low rates of lay were recorded for hens laying uncoupled sequences, and hens laying coupled sequences with rests. Low to negligible rates of lay were recorded in the remaining three categories (Table 15). It is evident, therefore, that the decrease in the percentage of birds in category a

noted in all strains is another factor responsible for the decline in the rate of lay. It is difficult to relate this decrease precisely to changes in the other categories, although broad correlations are possible. Babcock hens appeared to transfer from category a. to category d. The same was true of Ranger hens, but the percentage in category c. became more appreciable too as the hens aged (Table 14). Because of the negligible rate of lay of hens in this category (Table 15), this may explain the greater rate of decline in the rate of lay of Ranger compared to Babcock hens. Further, there were always more Ranger hens in category c than hens of any other strain. Ross I hens showed the most complex pattern. There was an appreciable percentage in category d at all times. The numbers in categories a. and d decreased in a near parallel manner. At the same time, the numbers in categories e, f and g appeared to increase (Table 14).

4.6.4

Determination of the First Period of Continuous Production

Birds were considered to be in continuous production from the onset of lay up to the initiation of a rest of seven or more days. The length of this period (in months) was calculated as 9.35 $\pm$ 5.72, 7.63 $\pm$ 3.86, 4.23 $\pm$ 3.93 (means $\pm$ standard deviation) for Babcock, Ranger and Ross I hens respectively. The duration of the rest period, from its initiation to resumption of production, was found to be (in days) 29.76 $\pm$ 44.86, 19.13 $\pm$ 23.68, 33.72 $\pm$ 54.69 (means $\pm$ standard deviation) for Babcock, Ranger and Ross I hens respectively. The distribution was completely skewed, however, such that the modal period of rest was 7.0d in all strains. This reflects the arbitrary division of periods of non-laying into 'uncoupled sequences' and 'rests'.

Fig 11

Survivors percent egg production of flocks of three strains of hens (Ross I; Ross Ranger, Babcock B300) kept in commercial-type windowless bird houses at the Roslin Outstation of the Poultry Research Centre.

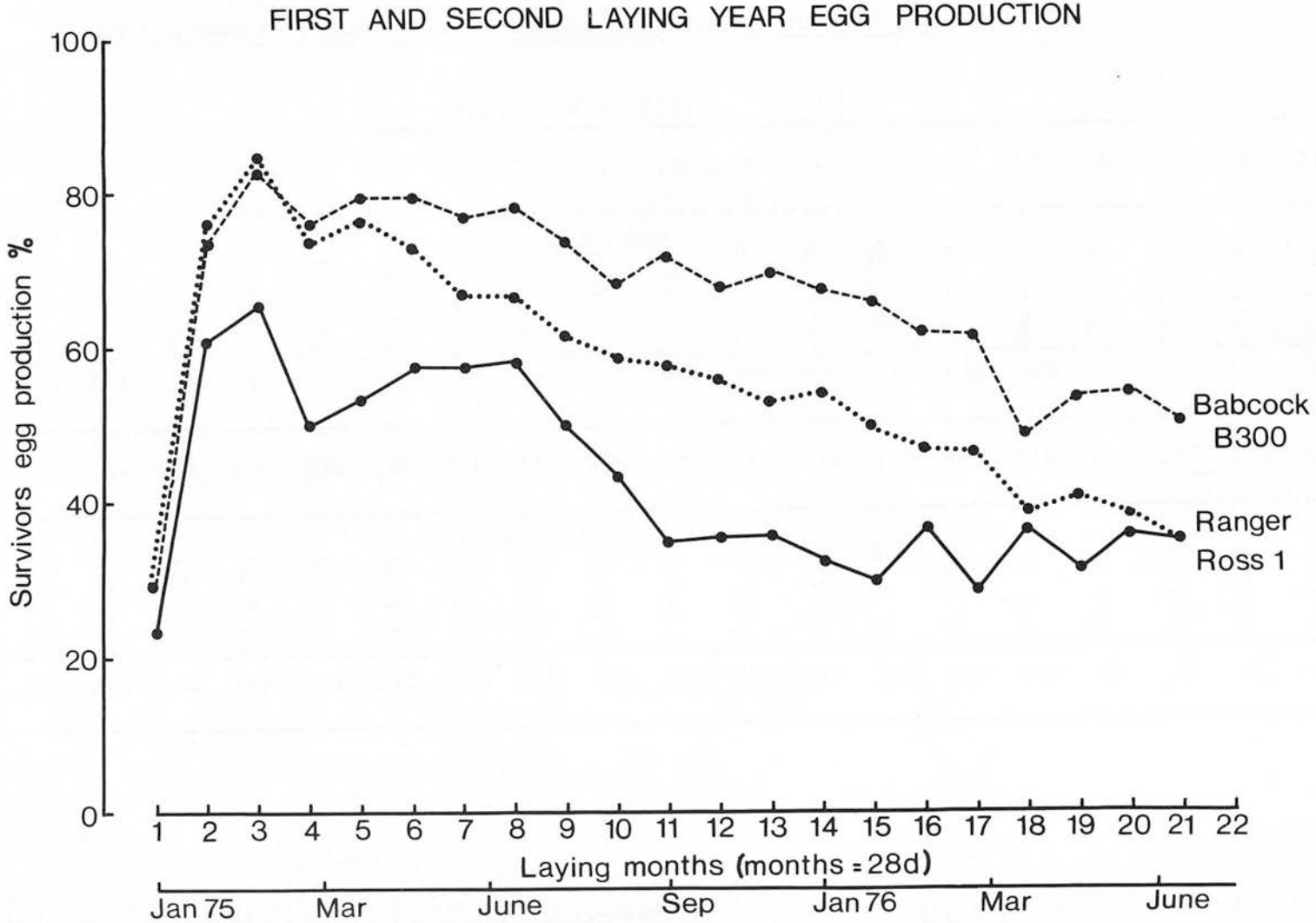

FIRST AND SECOND LAYING YEAR EGG PRODUCTION
Survivors egg production %
100
80
60
40
20
0
Babcock B300
Ranger Ross 1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Laying months (months = 28d)
Jan 75
Mar
June
Sep
Jan 76
Mar
June

Table 10

Monthly numbers of survivors in flocks of three strains of hen

STRAIN	'Month' (= 28d)																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	Date of Day One																					
	7.1.75	4.2.75	4.3.75	1.4.75	29.4.75	27.5.75	24.6.75	22.7.75	19.8.75	16.9.75	14.10.75	11.11.75	9.12.75	6.1. 76	3.2. 76	2.3. 76	30.3. 76	27.4. 76	25.5.76	22.6.76	20.7.76	17.8.76
BABCOCK	302	301	300	290	283	270	270	268	260	245	243	239	239	239	239	238	238	196	190	190	185	182
RANGER	305	305	305	304	286	286	284	283	281	280	279	279	277	125	120	117	116	116	116	114	108	107
	Date of Day One																					
	29.1.75	26.2.75	26.3.75	23.4.75	21.5.75	18.6.75	16.7.75	13.8.75	10.9.75	8.10.75	5.11.75	3.12.75	31.12.75	28. 1.76	25. 2.76	24.3.76	21.4.76	19.5.76	16.6.76	14.7.76	11.8.76	
ROSS I	269	266	266	256	246	244	241	240	220	219	218	218	218	218	218	127	127	81	81	81	81	

Table 11

Effect of Age on Variables of Sequence Length Distribution

			Ross	Babcock	Ranger
Modal Sequence Length	months	2-3	2	3	2
		7-8	2	3	2
		12-13	1	2	1
		17-18	1	1	1
Range of Sequence Lengths	months	2-3	1-21	1-58	1-64
		7-8	1-11	1-48	1-21
		12-13	1-8	1-38	1-14
		17-18	1-8	1-21	1-8

Table 12

All strains: Number of sequences for 100 birds of Length 'n'

Sequence Length 'n'	ROSS I				BABCOCK B300				RANGER			
	Months 2-3	Months 7-8	Months 12-13	Months 17-18	Months 2-3	Months 7-8	Months 12-13	Months 17-18	Months 2-3	Months 7-8	Months 12-13	Months 17-18
1	319.6	496.4	369.2	411.4	107.7	135	216	296.3	101.3	382	704.6	684.6
2	324.6	539.5	278.3	214.1	112.7	176.2	237.1	242.2	125.8	406.5	443.8	317.3
3	248.6	280.1	70.6	73.4	134.2	192.5	230.6	184	106.8	295.4	211.7	121.3
4	151.1	77.2	15.2	14.1	106.7	141.1	159.9	106.1	91.2	141.5	69.6	26
5	78.6	25	4.2	4.3	91.9	123.5	108.2	57.1	84.9	77.1	26.8	6.6
6	48.9	8.7	1.4	1.1	99	97.6	53.7	33.7	80.7	37.9	7.8	4.6
7	26.1	5.1			64.4	54.1	21.8	12.6	53.9	13.7	4.2	1.3
8	12.5	0.7	0.7	0.5	31.5	26.2	9.2	5.8	32.7	9.8	1.3	0.6
9	5.7	1.4			18.1	14.6	5.8	2.0	21.9	3.6	1.0	
10	4.6	0.35			14.1	12.8	5.1	1.7	16.3	3.6		
11	2.8	0.35			11.1	7.1	2.4	1.4	15.7	1.6		
12	2.1				15.1	5.8	2.4	0.7	11.4	0.6		
13	1.0				16.4	2.3	1.0	1.0	12.7	0.3		
14	0.7				8.4	4.0	1.3	0.7	13.7	0.6	0.6	
15	0.7				5.0	2.4	0.4	0.7	5.5	0.6		
16	1.4				5.4	0.7	0.7	1.0	5.2			
17	0.7				1.3	1.0	0.4		5.2			
18					1.0	1.0			3.6			
19					1.3	0.3	0.4		2.9			
20					4.0	0.3		0.3	1.3			
21	0.35				1.7	0.3		0.3	3.3	0.3		
22					1.3	1.0			1.6			
23					1.0	0.7			1.0			
24					1.3				1.6			
25					0.3				2.6			
26					0.7				1.3			
27									0.6			
28					1.3				2.0			
29					0.7	0.3			1.0			
30					1.3	0.3			1.6			
31					0.3				0.6			
32							0.4		1.0			

33		0.3			
34			0.7		1.3
35		0.7			1.0
36					
37					0.3
38				0.4	0.3
39			0.3		0.3
40					1.0
41					0.6
42		0.3			0.6
43		0.3			0.6
44		0.3			
45					0.3
46		0.7			
47					
48					0.3
49					0.3
50					
51					
52					
53					
54					
55					
56					
57					
58		0.3			
59					
60					
61					
62					
63					
64					0.3

Table 13

All strains: Percentage of Flock Laying Sequence of n Eggs

'n' Sequence Length	ROSS I				BABCOCK B300				RANGER			
	Months 2-3	Months 7-8	Months 12-13	Months 17-18	Months 2-3	Months 7-8	Months 12-13	Months 17-18	Months 2-3	Months 7-8	Months 12-13	Months 17-18
1	87.5	86.4	76.7	74.5	54.7	53.4	63.9	70.7	50.6	82.7	88.6	80
2	92.1	87.1	67.5	64.1	56.0	67.8	70.0	66.6	58.8	87.6	84.3	68
3	86.0	81.0	42.1	34.2	63.0	70.1	70.4	64.6	60.1	84.0	63.7	49.3
4	72.1	43.5	12.8	10.9	59.7	62.0	65.6	45.9	53.2	64.0	37.9	18.0
5	53.5	17.1	4.2	4.3	55.0	57.3	50.7	33.6	51.3	45.1	20.6	6.6
6	36.7	7.9	1.4	1.1	55.0	49.7	31.2	24.1	50.3	26.8	7.2	4.6
7	20.0	4.6			44.2	33.5	15.6	10.2	39.5	12.4	3.9	1.3
8	12.1	0.7	0.7	0.5	25.5	19.7	6.8	5.4	27.1	8.8	1.0	0.6
9	5.3	1.4			17.1	12.8	4.4	1.7	19.9	3.6	1.0	
10	4.6	0.4			12.4	11.4	4.8	1.4	14.3	3.3		
11	2.8	0.4			10.7	6.7	2.0	1.4	14.7	1.6		
12	1.7				13.7	5.0	2.4		10.4	0.6		
13	1.0				14.4	2.3	1.0	0.7	12.1	0.3		
14	0.7				8.4	4.0	1.0	1.0	13.1	0.6	0.6	
15	0.7				5.0	2.0	0.4	0.7	5.5	0.6		
16	1.4				5.0	0.7	0.7	0.7	5.2			
17	0.7				1.3	1.0	0.4		4.9			
18					1.0	1.0			3.3			
19					1.3	0.3	0.4		2.6			
20					4.0	0.3		0.3	1.3			
21	0.5				1.7	0.3		0.3	3.3	0.3		
22					1.3	0.7			1.6			
23					1.0	0.7			1.0			
24					1.3				1.0			
25					0.3				2.6			
26					0.7				1.3			
27									0.6			
28					1.0				2.0			
29					0.7	0.3			1.0			
30					1.3	0.3			1.6			
31					0.3				0.6			
32							0.4		1.0			

33		0.3			
34			0.7		1.3
35		0.7			1.0
36					
37					0.3
38				0.4	0.3
39			0.3		0.3
40					1.0
41					0.6
42		0.3			0.6
43		0.3			0.6
44		0.3			
45					0.3
46		0.7			
47					
48					0.3
49					0.3
50					
51					
52					
53					
54					
55					
56					
57					
58		0.3			
59					
60					
61					
62					
63					
64					0.3

Table 14

Percent of flock in each of seven categories of laying patterns during months 3 - 20 of lay

Key to strains: B = Babcock B300
Rn = Ross Ranger
Rs = Ross I

CATEGORY		a COUPLED SEQUENCES			b UNCOUPLED SEQUENCES			c REST		
STRAIN		B	Rn	Rs	B	Rn	Rs	B	Rn	Rs
	3	63.3	60.3	27.1	0	0	0	6.0	2.0	0
	4	0	0	14.1	1.4	1.0	0	3.4	3.9	5.1
M	5	65.0	55.2	15.2	0	0	0	0.4	7.3	0.8
O	6	57.6	39.9	18.4	0	0.7	0	5.9	7.0	0.4
N	7	50.9	30.6	27.8	0.4	0	0	7.7	7.4	0
T	8	64.6	40.3	23.3	0.4	0	0	2.2	8.8	0
H	9	47.8	23.8	13.2	0	0	0	3.1	8.9	1.4
S	10	45.3	23.9	4.1	0.4	0	0	3.8	11.1	4.1
	11	47.3	25.4	3.2	0.4	0	0	3.7	12.5	12.8
I	12	33.1	17.2	3.7	0	0.4	0	5.3	14.0	15.6
N	13	43.1	16.2	0.9	0	0	0.5	3.7	17.7	14.2
	14	38.5	18.4	1.4	0.4	0.8	0	3.1	31.2	17.0
L	15	31.0	9.2	-	0.4	0	-	4.6	30.8	-
A	16	24.4	5.1	8.7	0	0	0	5.8	30.8	0.8
Y	17	24.4	6.9	3.9	0	0	0.8	7.2	36.2	0
	18	16.8		7.4	0.5		0	4.1		0
	19	17.4		3.7	0		1.2	10.7		0
	20	13.0		3.7	0.5		0	9.8		1.2

Table 14 (cont.)

Percent of flock in each of seven categories of laying patterns during months 3 - 20 of lay

Key to strains: B = Babcock B300
Rn = Ross Ranger
Rs = Ross I

CATEGORY		d COUPLED & UNCOUPLED SEQUENCES			e COUPLED SEQUENCES + REST			f UNCOUPLED SEQUENCES + REST			g COUPLED SEQUENCES, UNCOUPLED SEQUENCES + REST		
STRAIN		B	Rn	Rs	B	Rn	Rs	B	Rn	Rs	B	Rn	Rs
	3	21.0	29.8	54.9	2.3	3.3	7.9	1.3	0.3	0.8	3.8	1.3	10.5
	4	91.7	91.4	50.4	0.7	1.0	11.7	0.7	1.0	2.7	2.1	2.3	16.0
M	5	26.1	42.7	47.1	4.9	1.0	22.5	0.4	0	1.6	3.2	0.7	12.7
O	6	32.5	57.7	63.5	1.8	1.0	5.7	0.4	0.3	1.6	1.8	0.3	10.2
N	7	38.5	64.8	55.6	0.4	1.8	7.9	0	0.7	0.8	2.1	2.5	8.3
T	8	28.0	54.1	62.1	1.9	1.4	4.6	0.7	0.4	0	2.2	3.5	11.3
H	9	40.7	69.4	63.2	4.2	0.4	6.4	1.5	1.4	1.8	2.7	5.0	14.1
S	10	39.2	62.1	49.3	7.3	3.6	9.1	1.6	1.4	2.3	2.4	7.1	31.1
	11	43.2	63.4	39.0	1.2	1.1	15.1	2.1	2.2	5.0	2.1	5.0	24.8
I	12	55.6	65.9	34.4	4.2	1.8	9.2	1.3	1.1	6.9	4.2	9.3	30.3
N	13	47.6	67.9	28.4	2.5	1.8	15.1	1.7	2.2	6.0	1.7	4.7	34.9
	14	47.3	61.6	26.1	3.8	0	13.3	2.5	1.6	3.7	4.2	6.4	38.5
L	15	52.3	70.0	-	2.5	2.5	-	2.9	1.7	-	6.3	10.8	-
A	16	54.2	71.8	35.4	2.1	4.3	19.7	3.4	5.1	9.4	10.1	11.1	26.0
Y	17	56.7	64.7	27.6	0.8	0	22.8	3.8	5.2	9.4	7.1	16.4	38.6
	18	57.1		34.6	7.7		17.3	3.6		6.2	10.2		42.0
	19	55.3		35.8	1.6		19.8	4.2		8.6	10.8		40.7
	20	57.8		35.8	4.3		12.3	1.1		3.7	13.5		46.9

Table 15

Mean Rates of Lay in Each Laying Category

	Category	Strain	Mean Rate (%)
a	Coupled Sequences	B	80.2
		Rn	73.6
		Rs	67.7
b	Uncoupled Sequences	B	44.1
		Rn	53.5
		Rs	33.0
c	Rest	B	0.8
		Rn	2.8
		Rs	0.8
d	Coupled & Uncoupled Sequences	B	68.8
		Rn	61.5
		Rs	55.1
e	Coupled Sequences & Rest	B	42.3
		Rn	36.2
		Rs	28.1
f	Uncoupled Sequences & Rest	B	13.5
		Rn	15.3
		Rs	13.1
g	Coupled & Uncoupled Sequences & Rest	B	35.9
		Rn	35.7
		Rs	31.7

Key to strains: B = Babcock B300

Rn = Ross Ranger

Rs = Ross I

Discussion

There was a 98% correlation between the egg laying records obtained automatically at King's Buildings and the manual records that were maintained in parallel. Data were screened for obvious errors (e.g. trip switch malfunction resulting in multiple records for one day), and editing was performed before the data were analyzed. In the case of the data recorded manually at Roslin, the data may have contained inaccuracies arising from operator error, but this error was probably small. All data were verified and corrected to eliminate errors arising out of the transposition of manual records to punch cards.

Both this study, and those of Heywang (1938) and Fraps (1955) , show that lag varies between successive eggs in a sequence. Lag is longer at the beginning and the end than it is in the middle of a sequence. How might this be brought about? Fraps (1965) theorized that lag could be caused by different times of LH release, as he had shown that the interval from injection of an ovulatory preparation to ovulation was constant. This has been confirmed by Wilson & Sharp (1973) who showed that the pre-ovulatory surge of LH was initiated early in the open period when the first ovum of a sequence was about to be ovulated, and at the end of the open period when the last ovum of a sequence was about to be ovulated. Pre-ovulatory surges initiated well into the open period were associated with intermediate members of a sequence. Fraps did not attempt to explain variable lag nor does his hypothesis take this into account. If, as hypothesized earlier, the pre-ovulatory LH surge can only be initiated once the follicle next due to ovulate can secrete sufficient progesterone to exert a positive feedback effect, variable lag must be a reflection of differences in the time required for successive follicles to reach maturity (i.e. attaining threshold capacity for secreting progesterone). Fraps & Dury (1942) showed that the first follicle of a sequence was much more sensitive to luteinizing hormone preparations injected at equal times before the expected ovulation than was any other follicle in the sequence. The first follicle of a sequence may therefore be considered

'supra' mature. This may be because more than 33 h elapses between ovulation of the terminal ovum of a sequence and ovulation of the first ovum of the following sequence, whereas within a sequence, the interval between consecutive ovipositions and therefore ovulations is 27 h or less. The first ovum therefore matures for at least 6 h longer than subsequent ova in a sequence. If this 'supra' mature first follicle is ovulated prematurely with LH or progesterone, the second follicle of the sequence matures prematurely and ovulates sooner than expected (Neher & Fraps, 1950). The time of maturation of the second follicle is not, therefore, predetermined. Rather, it is affected by the presence of the first follicle in the ovary. Wilson & Sharp (1976c) have also demonstrated that the maturation of the follicle next due to ovulate can be delayed by progesterone injections. It is, therefore, probable that the pre-ovulatory release of progesterone has a delaying effect on the ovulation of the second largest follicle in the hierarchy and hence affects lag in a sequence of eggs. One possible explanation for variable lag is the occurrence of larger or smaller progesterone pre-ovulatory surges before the various ovulations of a sequence. In support of this idea, Haynes et al. (1973) have reported that the first progesterone pre-ovulatory surge of a sequence is of much longer duration and is possibly elevated, compared to a pre-ovulatory surge of progesterone in the same hen occurring in the middle of a sequence. There are, however, few data available which enable one to compare successive pre-ovulatory surges in the same hen.

Wilson & Sharp (1976c) have shown that injections of progesterone affect the time of oviposition of an egg already in the oviduct as well as affecting the time of the next ovulation. Between 0 and 15 h after an ovulation, both may be delayed but progesterone injected 12 to 9 h before an ovulation (just before the pre-ovulatory release of endogenous progesterone would occur) will advance both oviposition of the egg in the oviduct, and ovulation of the next follicle. This effect of progesterone could, therefore, produce negative lag (see Fig 9 , 12 egg sequence).

Fraps (1955) presented a model illustrating the relationships between the ovarian follicles, ovulation and oviposition times in a 3 egg sequence. A key feature of this model was an interval of more than 24 h between successive follicles attaining minimum ovulability giving rise to lag within the sequence. The possibilty that short sequences are the result of a more protracted follicular maturation process is supported by the data on times of lay of first eggs in 1 and 2 egg sequences (Table 9). Such eggs are laid later in the day than first eggs of long (greater than 4) sequences. In a foregoing experiment (p.52) it was observed that the pre-ovulatory hormone surges corresponding to the first egg of a sequence were not always initiated at the onset of the open period i.e. darkness. Thus, the interval between succeeding follicles attaining minimum ovulability may be as great as 48 h in the case of a hen laying single egg sequences. Evidently follicles may mature to minimum ovulability within 24 h of each other in long sequences. It is possible that the pattern of lay in a long sequence may result from an interaction of the endogenous rate of follicular maturation and the regulating effect of one follicle on the ovulation of the subsequent follicle. As proposed above, this regulating effect may be mediated by the pattern of pre-ovulatory progesterone secretion.

The data presented above indicate that sequence length tends to shorten as hens grow older. In the case of birds laying coupled sequences, this may be due to a reduction in the length of the open period or to an increase in the time between successive follicles reaching the condition of minimum ovulability. Fraps (1955) has shown that the total lag in a sequence of four or more eggs depends closely on the length of the open period. As the total lag in 4-7 egg sequences was not found to be significantly shorter in months 10-11 of lay, compared to months 2-3 of lay, it seems more likely that the increased incidence of short sequences in old birds is caused by increased time between follicles reaching the ovulable condition. The question of follicular maturity is discussed fully elsewhere (p 97).

All departures from closed or coupled cycles can be

described as abnormal laying patterns, and the decision to classify the longer breaks between sequences as being due to 'uncoupled cycles' or 'rest periods' was quite arbitrary. In the first laying year, breaks in egg laying of 2-7 days are the predominant abnormal laying pattern in all strains. Longer breaks appear to be important causes of lost egg production in the Ross I hens at the end of the first laying year, and in Ranger hens at the beginning of the second laying year. Individual examples of short breaks are shown elsewhere (Table 19) and it is hypothesized (p 91) that the causes could be : internal ovulation, follicular atresia, failure to detect a laid egg (e.g. loss through the cage floor. Repetitive short breaks produced by these events would give rise to short sequences with very variable times of lay for the first egg of such sequences. Times of lay of first eggs of short sequences were found to be more variable than times of lay of first eggs of longer sequences (Table 9).

Evidence has been presented by Fraps (1965) that the times of lay of all eggs in sequences separated by a break of at least 5 days with no egg laid are identical with the times of lay in coupled sequences of the same length. Fraps (1965) also found that the ovaries of hens killed after laying the first egg of an 'uncoupled cycle' appeared normal, and no evidence was found of internal ovulation or follicular atresia. It is, however, not known whether the hens in Fraps' experiment underwent a rapid cycle of partial ovarian regression and redevelopment. Partial ovarian regression has been reported in incubating mallard ducks (Donham et al., 1977). After two days of incubation, the mean diameter of the largest follicle decreased from 30.8 mm to 6.6 mm and if the clutch of eggs was then removed, laying was normally resumed within 6 days. If laying hens can experience a similar rapid cycle of ovarian regression and development (possibly related to broodiness), then the normal times of egg laying in the sequence laid following a break of 5 or more days in laying may be the consequence of the establishment of a new follicular hierarchy. Thus, a better way of classifying hens showing irregular laying patterns may use as a basis the times of lay of the first eggs in a

a sequence following a break in laying.

Hens which stopped laying for long periods were identified by the program utilizing the 'rest' category. It is possible that the peak of out-of-lay or 'resting' Ross I hens in month 12 (Table 14) may be due to the occurence of the annual rest as defined by Lerner & Taylor (1942). Since no similar peak was observed in other strains, the inferior egg laying performance of the broiler breeder may in part be attributed to a short biological laying year. Nevertheless, the incidence of long rests was highest in Ranger hens (except in month 5) and this must have contributed to the lower productivity of these hens compared to Babcocks. The difference between Ranger and Ross I hens was that, in the case of the former, the numbers out of lay remained fairly stable from month to month and no peak incidence of out-of-lay birds could be distinguished.

4.7 Progesterone and LH secretion in relation to age, strain and egg production

In the hen, plasma LH (Sharp, 1975) and oestrogen (Senior, 1974) levels rise to a pre-pubertal peak, and then decline just before the onset of lay. It was decided to measure progesterone and LH in the same plasma samples as Sharp (1975) suggested that increased progesterone secretion is responsible for the pre-pubertal fall in LH levels.

Five females of each of the following strains were studied: Ross I (broiler breeder), a heavyweight strain; Ross Ranger, a mid-weight egg-laying strain; Babcock B300, a lightweight egg-laying strain. Of these, birds of the Ross I strain laid least well while birds of the Babcock strain laid the best. Birds of the Ranger strain laid almost as well as the Babcock strain (Fig 11). The experiment was also designed, therefore, to find out if there were any differences between the strains in patterns of the pre-pubertal plasma hormone levels which might be related to subsequent egg production.

5 ml blood samples were taken by venepuncture at weekly intervals, starting 8 weeks of age and continued to 29 weeks of age. Thereafter, blood was taken at approximately monthly intervals throughout the first laying year in order to establish the base-line levels of LH and progesterone during this period. Samples were taken in the afternoon as LH and progesterone levels should be at their lowest at this time in mature hens (Furr et al., 1973). Plasmas were assayed for LH and progesterone. Individual laying records were kept for all hens.

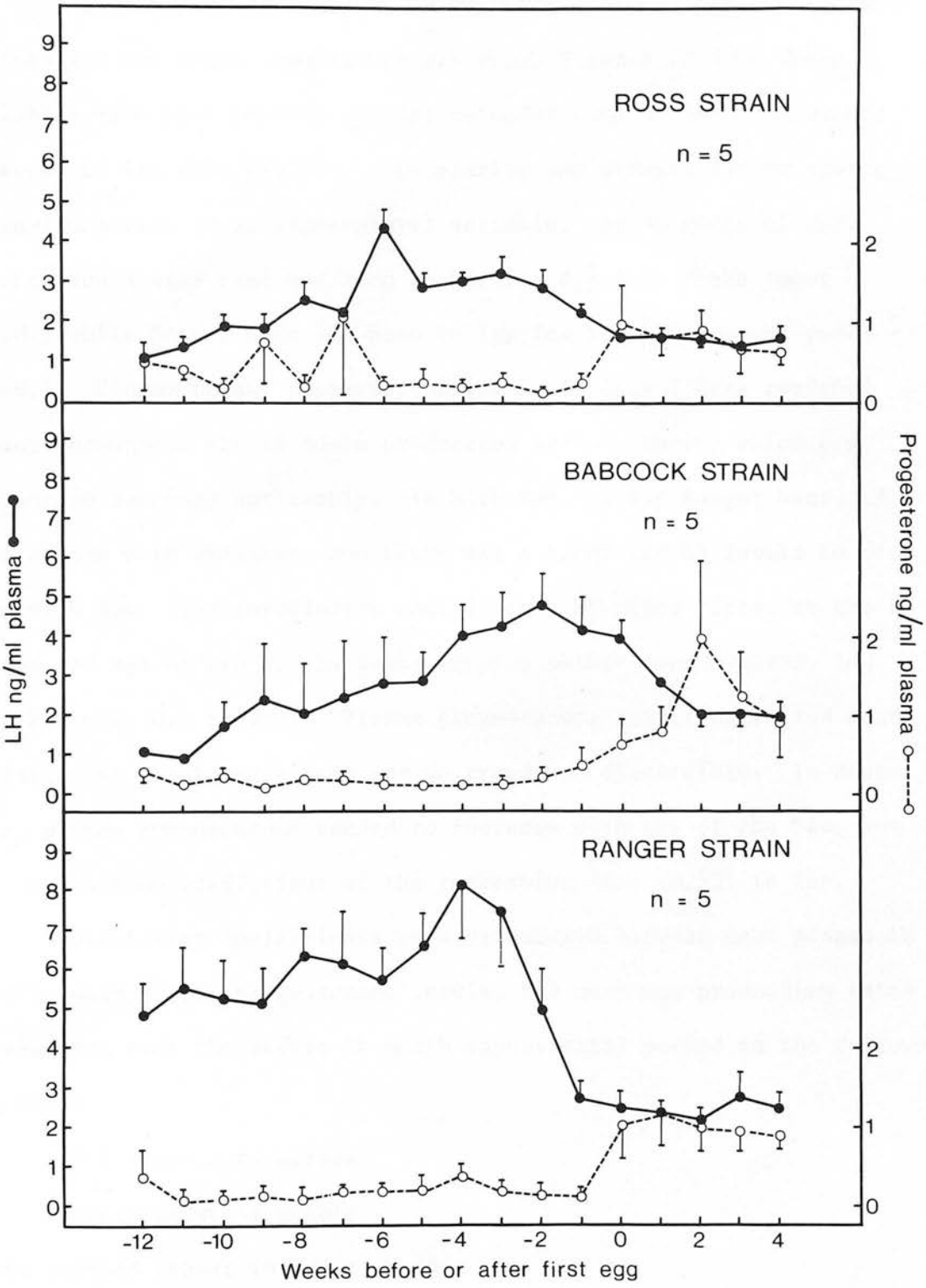
Plasma progesterone titres were found to be generally low, within the range 0 - 0.5 ng/ml plasma, from 8 weeks of age to just before the onset of lay. Occasional high values (> 1.0 ng/ml plasma) were noted

in some birds, mostly the Ross I strain. Coincident with, or just before the first egg was laid, plasma levels of progesterone rose and stabilised within the range 0.4 - 2.5 ng/ml plasma. They remained within this range during the first four weeks of lay (Fig 10). No evidence of a pre-pubertal peak of plasma progesterone was found.

A pre-pubertal plasma LH peak was, however, observed in all hens at between 17 and 19 weeks of age, but the timing of the peak with respect to the first egg differed between the strains (Fig 10). In the Ross I and Ranger hens, a clear peak was seen 6 and 4 weeks respectively before the first egg. In the Babcock strain, the highest plasma LH levels occurred 2 weeks before the first egg. Other between-strain differences were noticed. Mean pre-pubertal LH levels were of a similar order of magnitude in Ross I and Babcock hens, but in Ranger hens the mean LH levels were higher (Fig 10). Twelve weeks before the first egg, mean LH levels of 1.1 ng/ml plasma were observed in Ross I and Babcock hens, and mean LH levels of 4.8 ng/ml plasma were observed in Ranger hens. Mean plasma levels of LH of 2.5 - 5.0 ng/ml were found in Ross I and Babcock hens around the time of the pre-pubertal peak, while in the Ranger hens, the range of mean plasma LH levels around the time of the peak was 5.5 - 8.2 ng/ml (Fig 10). In both Ross I and Ranger hens, plasma LH titres fell to lower levels before plasma progesterone levels increased and the first egg was laid. These lower levels (c. 1.5 ng/ml plasma, Ross I; c. 2.5 ng/ml plasma, Ranger) remained steady during the first 4 weeks of lay. In Babcock hens, LH levels had only fallen slightly from the peak levels when increased plasma progesterone was seen and the first egg was laid. Thereafter, plasma LH continued to fall reaching the steady state of about 2 ng/ml in the second week of lay (Fig 10). Although the decline in LH levels from the pre-pubertal maxima to in-lay levels was statistically significant in all strains ($P < .02$; paired

Figure No. 10

CONCENTRATIONS OF LUTEINIZING HORMONE AND PROGESTERONE DURING THE SEXUAL DEVELOPMENT OF THE HEN
(means ± SEM)



t-test), the increases in plasma progesterone levels at the onset of lay were only statistically significant in Ranger hens ($P < .02$; paired t-test)

The mean plasma levels of LH and progesterone, percent egg production and hen house temperature are shown Figures 12 & 13. These variables have been plotted against calendar time as well as number of weeks in lay (cf. Fig 10) to clarify any effects due to ageing or environmental (e.g. temperature) variable. At 30 weeks of age, Babcock and Ranger hens had been in lay for 8 ± 1.06 weeks (mean $\pm$ S.E.M.) while Ross I hens had been in lay for 5.4 ± 0.8 weeks (mean $\pm$ S.E.M.). Plasma LH and progesterone levels in Ross I hens remained steady throughout the 15 month production period, during which egg production declined noticeably. In both Babcock and Ranger hens, LH levels were more variable, and there was a trend for LH levels to decline with age. The correlation coefficients of lines fitted to the LH values and age of hen by the least squares method are, however, low (0.2 Babcock, 0.6 Ranger). Plasma progesterone levels exhibited much variation in the Babcock hens and no trend was discernible. In Ranger hens, plasma progesterone tended to increase with age of the hen, but the correlation coefficient of the regression line (0.53) is low.

Correlation coefficients were calculated between mean plasma LH levels, mean plasma progesterone levels, and mean egg production rates of each hen over the entire 14 month experimental period in the following ways:

1. With respect to strain
2. Irrespective of strain

These results appear in Table 16

Further correlation coefficients were calculated, comparing the

mean plasma LH levels and the egg production of all birds of one strain for each of the 14 laying months studied. These were: Ross, r^2 = 0.027; Babcock, r^2 = 0.005; Ranger, r^2 = 0.484. The correlation coefficients of mean plasma progesterone and egg production, likewise calculated were: Ross, r^2 = 0.013; Babcock, r^2 = 0.052; Ranger, r^2 = 0.323.

In all hens, LH levels appeared to be depressed at 50 weeks of age. At this time (August, 1975) high maximum temperatures (Fig 12; mean maximum = 25.3^{o}C) were recorded in the hen house. This depression of plasma LH levels was significant ($P < 0.05$; paired t-test) compared to the LH levels found at 38 weeks of age in all strains. Egg production did not, however, decline in association with this depression in LH levels (cf. Figs 13 & 11).

Figure 12

Hen house mean monthly maximum and minimum temperatures. All strains of hen studied (Figs 10, 11 & 13) were housed together.

HEN HOUSE MEAN MAXIMUM & MINIMUM TEMPERATURES

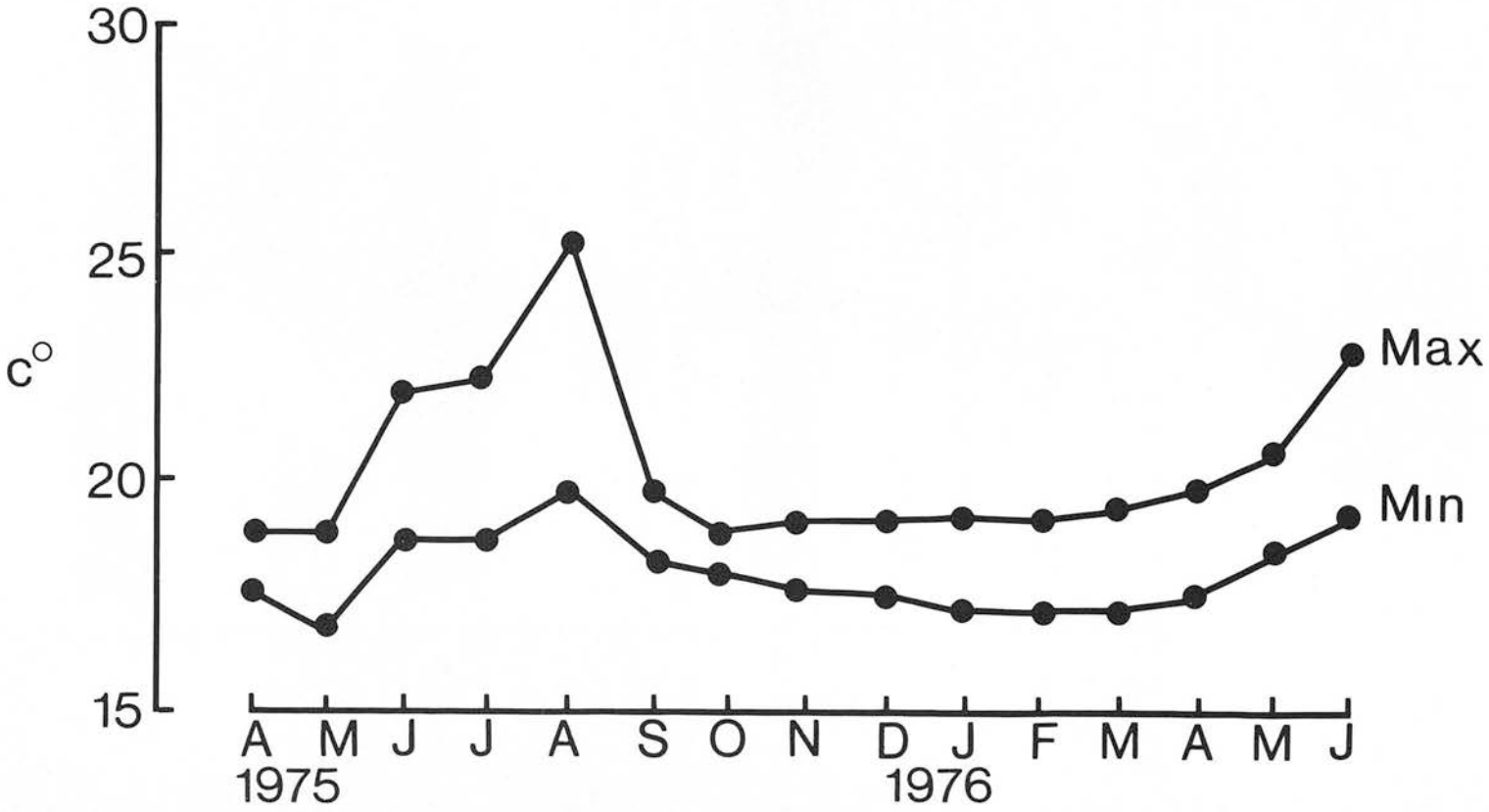


Figure 13.

Mean plasma LH and progesterone concentrations (°) in hens throughout the first laying year.
Shaded areas represent the mean monthly egg production of the five hens of each strain studied.

MEAN PLASMA LH AND PROGESTERONE CONCENTRATIONS IN HENS THROUGHOUT THE FIRST LAYING YEAR

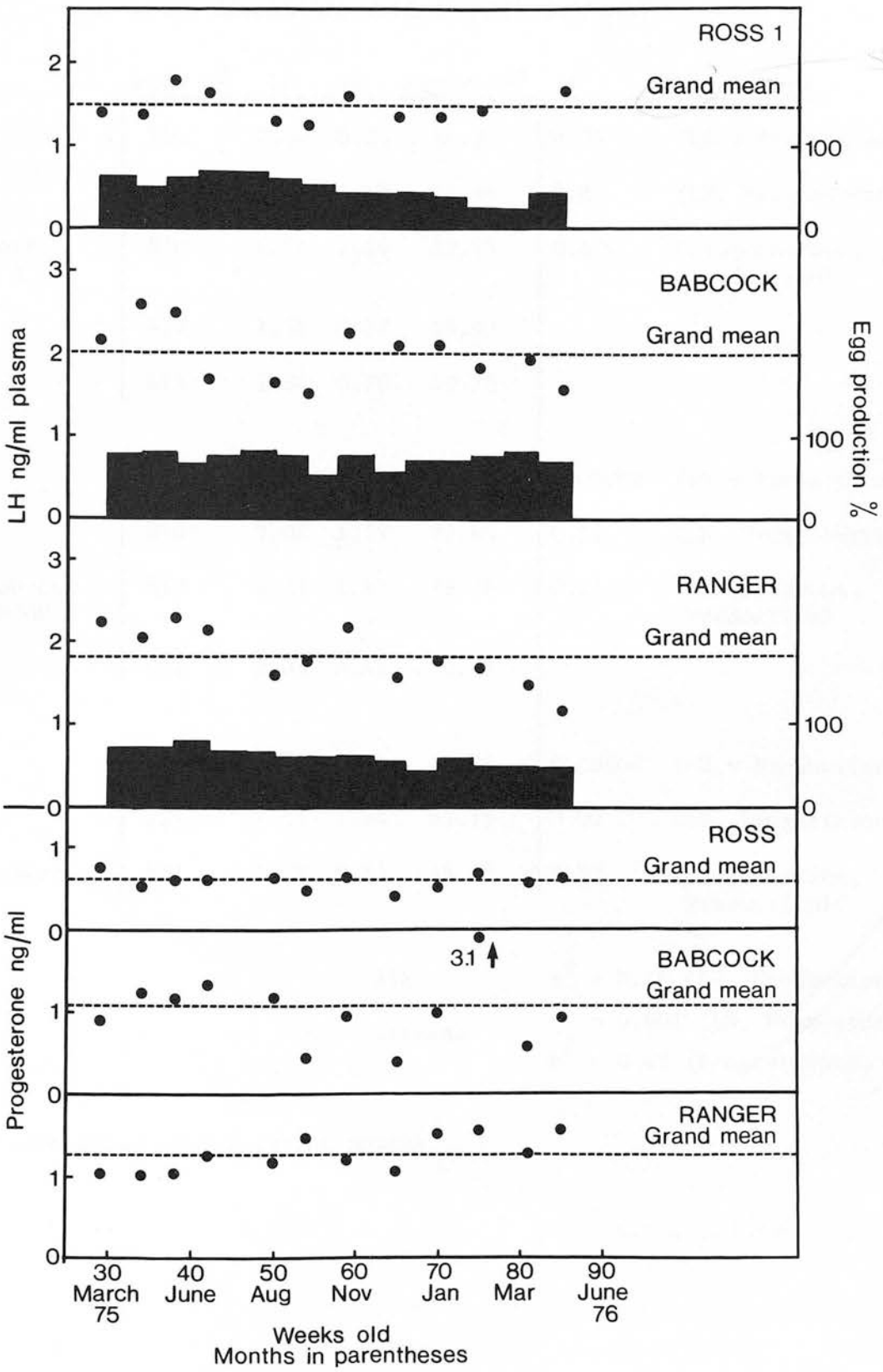


Table 16

Mean LH plasma levels, mean progesterone plasma levels and mean egg production over 14 laying months

	Bird N°	LH	P4	Egg Prodⁿ	r^2	Correlation
Ross I	806	1.36	0.25	46.9	0.77	(LH v Production)
	807	1.79	1.10	61.44	0.8	(LH, Progesterone)
	810	1.56	1.14	53.29	0.68	(Progesterone, Production)
	812	1.36	0.22	39.92		
	814	1.34	0.28	49.73		
Babcock B300	3033	1.78	0.77	65.19	0.00004	(LH v Production)
	816*	2.02	1.75	77.65	0.51	(LH, Progesterone)
	817	1.34	1.87	75.36	0.25	(Progesterone, Production)
	821	2.83	0.48	72.62		
Ranger	828	2.16	2.19	66.26	0.00007	(LH v Production)
	829	1.35	1.84	62.15	0.01	(LH, Progesterone)
	831	1.79	0.54	58.44	0.85	(Progesterone, Production)

All strains

$r^2 = 0.26$ (LH, Production)

$r^2 = 0.001$ (LH, Progesterone)

$r^2 = 0.45$ (Progesterone, Production)

* calculated over 5 laying months

Discussion

The hypothesis (Sharp, 1975) that increased progesterone secretion in the 2-3 weeks preceding lay is responsible for the reduced pituitary response to LH-RH, and hence for the decline in basal plasma LH levels from the pre-pubertal maximum is not supported by the present observations. In two strains, Ross I and Ranger, plasma LH levels had fallen to basal values typical of laying hens before plasma progesterone increased. Although the decline in basal plasma LH levels from the pre-pubertal peak in Babcock hens occurred in synchrony with an increase in plasma progesterone, the relationship between increased progesterone secretion and the onset of lay held true in this strain as in the other two strains. Thus, in Babcock hens, the first egg was laid before basal plasma LH levels had decreased to typical adult values. This suggests that the mechanism governing development of the ovarian follicles is independent of the mechanism which restrains basal LH secretion in the final stages of sexual maturity. This suggestion is supported by different mean latencies of the onset of lay measured from the time of peak pre-pubertal plasma LH levels in each strain: Ross I, 6 weeks ; Ranger, 4 weeks ; Babcock, 2 weeks. Furthermore, if the fall in basal plasma LH from pre-pubertal levels is due to decreasing pituitary responsiveness to LH-RH (Cunningham et al., 1973 ; Wilson & Sharp, 1975c), the degree of pituitary responsiveness is not critical to the ovulatory process. Possibly, it is the maturation of the progesterone-induced positive feedback mechanism governing the pre-ovulatory release of LH that is important. Wilson & Sharp (1975c) studied the development of this positive feedback mechanism. Their mean data indicate that it is first active at 18.3 weeks of age when basal plasma LH levels are rising, and fully developed at 23.1 weeks of age just prior to the onset of lay. They have also shown, in a longitudinal study on individual hens, that the mechanism can mature in as little as two weeks. The difference between the hormone profiles of the immature Babcock hens, and those of the other two strains indicate that the positive feedback mechanism

matures quickly in the Babcock strain and , possibly, slowly in the other two stains. In the Ross I strain, the late onset of sexual maturity must evidently be attributed to the restricted feeding regime utilised expressly to this end (Blair, 1972).

Plasma oestrogens reach a pre-pubertal maximum in the hen after which the levels fall over a 2-3 week period before the onset of lay (Senior, 1974 ; Peterson & Webster, 1974). While administration of oestrogen depresses plasma LH levels in the ovariectomized hen, and an oestrogen/progesterone combination is even more effective (Wilson & Sharp, 1976b), oestrogen enhances the pituitary response to LH-RH in vitro and in vivo. (Bonney & Cunningham, 1974). In the maturing hen, it is possible that falling oestrogen levels could account for reduced pituitary responsiveness to LH-RH, and hence falling LH levels in the 2-3 weeks before the onset of lay. This suggestion must only be tentative as the oestrogen measurements on maturing hens referred to above relate to different strains to those used in the present experiments. In view of the between strain differences in pre-pubertal LH secretion patterns reported here, it cannot be assumed that the timing of the pre-pubertal peak and subsequent decline of plasma oestrogen levels parallels the LH secretion pattern in all strains of hen.

It is not clear why high temperatures appeared to depress LH levels but not egg production. Possibly, only basal LH levels were depressed but not the cyclic pre-ovulatory release of LH. Alternatively, basal LH levels may fluctuate within a large range of values without affecting egg production. In support of this idea, one may cite the observation that plasma LH levels declined from 4.0 ng/ml to 2.0 ng/ml in Babcock hens during the first two weeks of lay, during which time hens were attaining maximal egg production rates. Also, in Ross I and Babcock hens, there was no significant correlation between egg production and LH over the 14 laying months studied. However, it could be argued that the lack of correlation is due to short-term fluctuations of basal LH levels in individual birds. A more precise determination of basal plasma LH levels would require a) sampling at more frequent intervals, and b) the use of larger groups of animals (group size in these experime-

nts was 3-5 birds). It is also possible that the fluctuating basal concentrations of LH in the plasma can be attributed to varying amounts of cross-reacting substances in assay plasma samples (see p.95).

The long-term study on laying hens did not reveal a simple, uniform relationship in all strains between plasma LH and progesterone. This might be expected to exist as LH is steroidogenic in laying hens (Shahabi et al., 1975b), although other factors such as prolactin affect progesterone secretion in the turkey hen following an injection of LH (Camper & Burke, 1977). In the Ranger hens, which showed the lowest correlation between LH and progesterone plasma levels, there was a tendency for plasma progesterone levelsto increase as the birds aged, but such a trend could not be detected in the other two strains. This is of interest in view of the observation by Furr (1973) that plasma progesterone levels are higher in old, as compared to young, laying hens but the strain of hen studied was not mentioned.

4.8 The Relationship between ovarian growth, structure and strain and age of bird, and the relationship of these variables to the rate of lay

Attention was drawn earlier to the inferior egg-laying performance of broiler (meat-type) hens. These hens never attain the rate of lay of egg-type strains even at their peak laying period, between 6 and 8 weeks after the onset of lay. Secondly, the rate of decline of egg laying with age is more marked in broiler type hens compared to egg-type hens. The rate of recruitment of ovarian follicles into the hierarchy of yolky follicles might be an important factor affecting the rate of egg laying. The observations of Jaap & Clancy (1968) that the ovaries of broiler hens contained more yellow, yolky follicles (6.5/ovary) than the ovaries of an egg-producing strain of the same age (5.6/ovary) suggests that a reduced rate of recruitment of follicles is not the underlying cause of the broiler hen's poor egg productivity. However, the broiler hens used in Jaap & Clancy's experiment were fed ad libitum whereas it is normal commercial practice to restrict food intake of broiler hens as described by Blair, MacCowan & Bolton (1976). It was decided to repeat Jaap & Clancy's (1968) experiment using a commercial egg-laying strain (Babcock B300) and a commercial broiler breeder (Ross I) fed a restricted diet.

Groups of birds from each strain were killed when their rates of lay were at a peak (26 weeks of age) and when egg production was falling (at 82 weeks of age). The two groups of 82 week old birds were subdivided into good (> 50% egg production) or poor (< 50% egg production) egg producers. Group or sub-group sizes ranged between 23 and 26.

After death, the carcass, ovary and oviduct were weighed.

The yellow yolky follicles were counted and weighed individually, and the remaining ovarian weight was noted. The number of post-ovulatory follicles was also noted. The egg production of individual birds was calculated from the number of eggs laid in the 28 days before death.

Measurements of follicular weights and numbers, ovarian weight, number of post-ovulatory follicles, oviduct weight, body weight and egg production for the six groups or sub-groups of birds were compared by an analysis of variance for strain and age differences, and differences between the sub-groups of old birds.

The measurements obtained from birds killed in this experiment are summarized in Table 17. Babcock hens produced more eggs than Ross I hens ($P < .001$) and all young hens were superior layers to all old hens ($P < .001$). The division between old good layers and old poor layers was well drawn: 69% vs 36% ($P < .001$).

The number of yellow, yolky follicles and their total weight did not differ in the two strains, nor did it differ between good and poor producing old birds. There was, however, a highly significant difference ($P < .001$) in yellow, yolky follicle number between all young birds (mean no. = 7) and all old birds (mean no. = 5) yet the total weight of these follicles was significantly greater ($P < .001$) in the old birds (mean wt. = 41.8g) than in young birds (mean wt. = 33.6g). There was no significant difference in the weight of all yolky follicles between good and poor producing old birds.

The weight of the ovary remaining after removal of the yellow, yolky follicles did not differ with strain, age or production rate. No strain or age differences were detected in

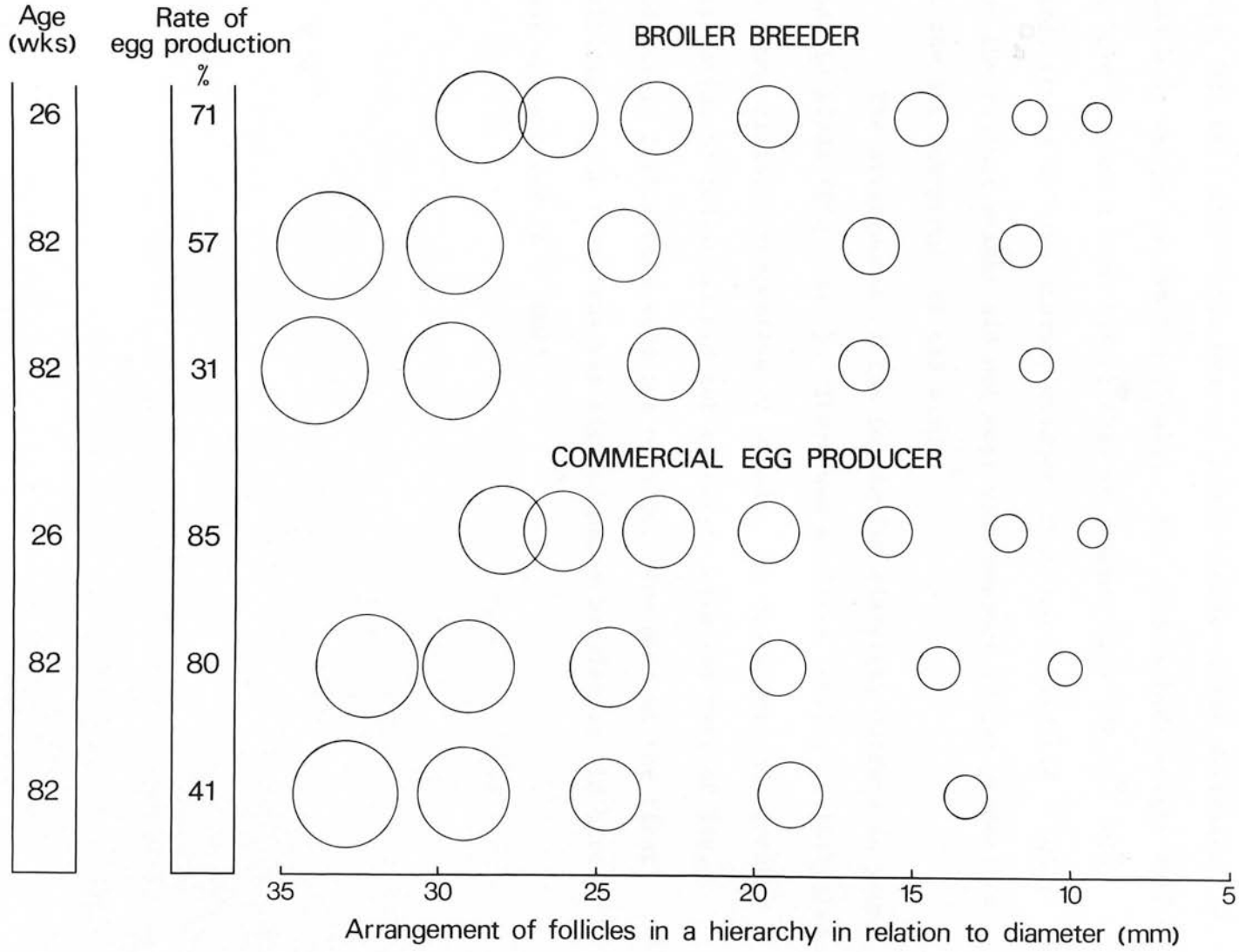
Table 17

Relationships between gross ovarian morphology and the rate of lay in broiler breeder and commercial egg-laying strains of domestic hen (means + SEM)

Type of hen	Age (wks)	Number of hens	Body wt. (kg)	Rate of egg production (%)	Number of yellow-yolky follicles	Total weight of yellow-yolky follicles (g)	Weight of ovary without yellow-yolky (g)	Number of post-ovulatory follicles	Oviduct weight (g)
Broiler Breeder									
Young	26	26	2.43±.036	71±2.15	7±.20	33.5±1.4	6.7±.33	4±.21	49.6±1.70
Old good layers	82	18	3.49±.044	57±2.6	5±.24	42.3±1.67	7.2±.38	3±.25	59.7±2.05
Old poor layers	82	20	3.54±.041	31±2.46	5±.22	40.1±1.6	6.7±.36	3±.24	60.6±1.91
Commercial egg producer									
Young	26	23	1.43±.038	85±2.3	7±.21	33.6±1.48	6.3±.33	3±.225	41.1±1.8
Old good layers	82	21	1.81±.04	80±2.39	6±.22	41.4±1.55	7.0±.36	4±.23	57.3±1.89
Old poor layers	82	20	1.99±.04	41±2.46	5±.22	43.5±1.60	7.4±.36	3±.24	58.1±1.94

Calculated for each bird from the number of eggs laid during the 28d preceding death

Fig. 14 A diagrammatic comparison of the arrangement, size and numbers of yellow-yolky follicles in young (26 weeks) and old (82 weeks) hens of a broiler breeder strain and a commercial egg producer strain. The 82 week-old hens were divided into good and poor layers: the poor layers laid at approximately half the rate of good layers. Each open circle represents the mean size of a follicle in a given position in the follicular hierarchies of between 18 and 26 hens. The follicular diameters are 1/2 of the scale size. The rate of egg production was calculated for each bird from the number of eggs laid during the 28d preceding death.


Age (wks)
Rate of egg production %
BROILER BREEDER
26
71
82
57
82
31
COMMERCIAL EGG PRODUCER
26
85
82
80
82
41
35
30
25
20
15
10
5
Arrangement of follicles in a hierarchy in relation to diameter (mm)

the number of post ovulatory follicles, but significantly more ($P < .001$) were found in old, high-producing birds as compared to the old, poor producers.

Ross I birds tended to have heavier oviducts than Babcock hens, but this difference was not proportional to the difference in mean body weight of the two strains. The oviduct/body weight ratio is less in Ross I hens (18.4)* than in Babcock hens (29.8)*. Old birds tended to have heavier oviducts than young birds ($P < .001$) but the oviduct weight did not vary with respect to egg production in the two sub-groups of old birds.

The arrangement of the follicular hierarchy differs in young and old birds (Fig. 14). There was a closer ranking of follicles in young birds, irrespective of strain. In old birds, the greater separation of follicles did not correlate with the rate of lay, and strain differences were not obvious. The mass of the first follicle of the hierarchy was significantly heavier in old birds than young birds ($P < .001$).

* $\times 10^{-3}$

4.9 Rates of Growth of Yellow Yolky Ovarian Follicles in Relation to Age and Rate of Lay

Fat-soluble dyes given either orally or intravenously are deposited in the yolk as a discrete band (Warren & Conrad, 1939; Lacassagne, 1970; Gilbert, 1970). This observation has been exploited by several authors to show that the final rapid growth of yellow yolky ovarian follicles lasts for between 7 and 11 days (review: Gilbert, 1971). The duration of the final rapid growth phase reaches a maximum 2 months after the first egg when hens are laying at peak rates (Gilbert, 1972). It was considered worthwhile to compare the duration of this rapid growth phase in young, old good and old poor-egg producing hens in relation to the different arrangements of follicles in the hierarchies of old and young hens described in the previous section.

A single gelatin capsule containing a measured dose of Scarlet R was fed to each bird. The rate at which dye disappeared from the yolks of successive eggs was taken to reflect the rate of growth of the yellow, yolky follicles. Eggs were collected and counted over a two-week period following administration of dye, hard-boiled, and sliced to determine if dye had been deposited in the yolk. The diameters of dye rings were recorded. Birds were killed when their eggs contained no more dye and the number of yellow, yolky follicles in their ovaries was recorded.

At post-mortem, the ovaries of the hen fed dye capsules resembled those of hens described in the previous section in terms of the mass and diameter of the largest follicle. The number of yellow yolky follicles was greater in the young birds (26 week old) than in the older (52 week old) birds. The oldest birds had as many yellow, yolky follicles as the youngest birds. The number of days

to clear dye, the number of dyed eggs laid, and the rate of lay are shown in Table 18.

In all hens, yolks were recovered where the diameter of the smallest dye ring was less than 5mm. The mean number of eggs containing dyed yolks was not significantly different from the mean number of yellow yolky follicles found in the ovary at post-mortem in the case of 113 week old hens. In the 26 week old and 52 week old hens, however, the mean number of dyed eggs recovered was greater than the number of yellow, yolky follicles found in the ovaries.

Inspection of the follicular hierarchies at post-mortem provided no evidence of gaps in the hierarchies (Table 19). Using the measurements of dye-rings in successive eggs, it was possible to construct a representation of the follicular hierarchies of hens at the time of dye administration and assimilation. Such hierarchies consisted of a regular graded series in all the 26 week old and in over 90% of the 52 week old hens, but gaps were evident in many 113 week old birds (Table 19). It was established that birds displaying such gaps had normal numbers of yellow, yolky follicles at post-mortem.

Table 18

Egg production, ovarian structure and rate of disappearance of dye from the yolks of eggs laid by hens of a commercial egg producing strain of different ages after feeding each hen a capsule containing a fat soluble dye (means ± SEM)

Age in weeks	26	52	113
Number of hens	17	10	16
Egg production (%)[1]	86.1 ± 2.1[a]	78.4 ± 3.0[a]	53.3 ± 4.5[b]
Number of eggs with dyed yolks collected from each hen	9.1 ± 0.2[a]	7.4 ± 0.3[b]	5.2 ± 0.6[c]
Days to the disappearance of dye from eggs laid	11.5 ± 0.2[a]	10.8 ± 0.1[a]	11.6 ± 0.5[a]
Number of yellow-yolk follicles in the ovary of each hen	5.5 ± 0.2[ab]	4.6 ± 0.2[b]	5.5 ± 0.2[a]
Weight of largest ovarian follicle (g)	12.7 ± 0.2[a]	15.7 ± 0.6[b]	16.3 ± 0.5[b]
Diameter of largest ovarian follicle (mm)	28.2 ± 0.2[a]	31.4 ± 0.6[b]	32.0 ± 0.4[b]

Means followed by different superscripts differ significantly ($P < 0.01$, Mann-Whitney U-test)

1. Calculated for each hen from the number of eggs laid during the 28 days before death

Table 19

Examples of the diameters (mm) of dye rings deposited in the yolks of eggs laid by a commercial egg producing strain of different ages after feeding each hen a capsule containing a fat soluble dye

	Days after dye administration													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	–[a]	30	26	23	20		15	9	6	3	2	–		–
		29	27	22	19	17		9	7	5	4	2	–	–
	–	30	29	25	21	17	12	7	5	4	1	–	–	
26 weeks old		–	29	27	20	15	12		5	3	1	–	–	
	–	28	26	22	20	15	12	7	4	4	1	1	–	
		32		26	23	20	14	10	9		2	2	–	–
	–		28	22	22	17	12	7	5	4	–	–		
	–	30		29	23	21	18	13	8	5		4	1	–
	–		30	26	22	14		7	4	4	1		–	–
			32	26	22	15		11	6	1	–	–		
		30	30	25	22	16	7		6	2	1		–	–
	–		28	22		13	6		4	3	1	–	–	–
52 weeks old			34	25	22	17	10		9		2	–	–	–
	–	34	28	25		20	18	11	7		2		–	–
	–	32	30	25	22		14	9	4		1	–	–	–
	–		33	29	22	17		10	6	1		–	–	–
	–			34		25	20		14		5		2	1
		–	28		24		17	10		6	1	–	–	–
	–	34		30	25	22					4		2	1
113 weeks old			33									–		
				30		22	11					–	–	–
			32		30	20			7		5	–		–
	–		32	28		23		16		5	4	2		–
			33		30			16			7	–		

[a] a dashed line indicates that there was no dye in the yolk

4.10 The sensitivity of the hypothalamus, the pituitary and the ovary in old and young laying hens

The functional integrity of the hypothalamo-pituitary-ovarian axis is essential to ovulation. It may be possible to explain declining rates of lay in old birds in terms of the LH-RH system becoming less sensitive to progesterone stimulation, the pituitary responding less to LH-RH or the ovary becoming less sensitive to LH. These possibilities were examined in the experiment described below.

10 and 24 month old Babcock B300 hens were used, housed in the automatic egg recording cages at King's Buildings. The egg production figures quoted below were calculated on the basis of production in the 28 days preceding the experiment. Three separate experiments were performed.

4.10.1 LH-RH stimulation test

Pituitary responsiveness to LH-RH was investigated by the administration of either 0.9% saline (controls) or 20 μg/kg body weight of synthetic LH-RH (Hoechst Pharmaceuticals Ltd). One blood sample was withdrawn 10 min prior to injection, and another immediately before injection, into the brachial vein. Post-injection blood samples were obtained 6 and 12 minutes after injection. All injections were given in the afternoon, after an egg had been laid, when plasma LH concentrations would be low (Wilson & Sharp, 1973). Plasmas were assayed for LH concentrations.

Plasma concentrations of LH were elevated 6 minutes after injection of LH-RH and a further small elevation was evident 12 minutes after injection (Fig 15). This elevation was highly significant compared with the pre-injection plasma LH concentrations and with the post-injection LH values of the saline-injected controls ($P < .001$; paired t-tests). The mean change in

plasma LH concentrations in young birds, 1.65 ± 0.16 ng/ml (mean ± SEM) was not significantly greater than the mean change seen in old birds (1.53 ± 0.16 ng/ml). The egg production of the group of young experimentals was 82% ± 1.43% (mean ± SEM), while for the old birds it was 61.6% ± 3.14%. These differences were highly significant ($P < .001$; unpaired t-test).

4.10.2

Responsiveness of the progesterone-induced positive feedback mechanism

The sensitivity of the brain and the LH-RH system was investigated by administering either an intramuscular injection of arachis oil (controls) or an i.m. injection of 0.5 mg/kg progesterone dissolved in 0.5 - 0.6 ml arachis oil (experimentals). One blood sample was taken just prior to injection and a further 6 at 40 minute intervals thereafter. The dose of progesterone chosen has been shown to reliably stimulate LH release from the pituitary gland by positive feedback (Wilson & Sharp, 1975). All injections were given in the afternoon, after an egg had been laid, when plasma LH concentrations would be low (Wilson & Sharp, 1973). Plasmas were assayed for LH.

Plasma LH concentrations started to increase 80 mins after injection and thereafter increased steadily to reach significantly elevated ($P < .001$; paired t-test) values at 2.6h post injection in both young and old hens. These maximum values were maintained for the remainder of the experiment (Fig. 16). The maximum change in plasma LH concentrations in old birds (1.19 ng/ml ± 0.11 SEM) was significantly less ($P < .05$) than the maximum change seen in young birds (1.44 ng/ml ± 0.16 SEM). Egg production figures for the young and old birds were 79.3% ± 1.82% and 68.1% ± 4.2% (means ± SEM) respectively, and were significantly different ($P < .01$; unpaired t-test).

4.10.3

Ovarian response to ovine LH

In a preliminary study, hens were injected intravenously with 10, 20 or 50 μg/kg NIH-516 ovine LH between $4\frac{1}{2}$ to $9\frac{1}{2}$ hours after an oviposition in the middle of a sequence of eggs. This corresponds to between 4-9h after an ovulation when the largest ovarian follicle would not be fully mature. The same doses were given to hens $4\frac{1}{2}$ to $7\frac{1}{2}$ hours after the terminal oviposition of a sequence, corresponding to a period 30-33h after an ovulation when the ovary contained a mature, ovulable follicle. Control hens were given an i.v. injection of saline. Blood samples were taken just prior to injection and at 10, 30, 60 and 90 min intervals thereafter. Plasma was assayed for progesterone.

The results of this study (Fig. 17) indicated that the smallest dose of LH that would reliably produce a discrete elevation of plasma progesterone concentrations was 20 μg/kg body weight. This dose was used in the main study where young and old birds were injected as described above at the two different stages of the ovulatory cycle, and plasma was assayed for progesterone.

Plasma progesterone concentrations rose within 10 min. of injection in all birds injected with LH at all stages of the ovulatory cycle. Peak values were attained in the majority of birds 30 minutes after injection, and declined thereafter to base-line levels. In 2 birds injected 30-33h after an ovulation, plasma progesterone concentrations increased throughout the post-injection sampling period, a response typical of hens given 50 μg/kg LH 30-33h after an ovulation in the pilot study. The rise in plasma progesterone concentrations observed at 30 mins post-injection was highly significant ($P < .001$; paired t-tests) when compared to pre-injection values, or values in controls 30 min. post-injection.

The response in young birds was identical when the largest ovarian follicle was mature or immature. No significant differences were observed in base line plasma progesterone concentrations, the maximum change or the mean change in plasma progesterone concentrations, or the areas under the curves. There was no significant difference in the egg production rates of the two groups of young hens injected at different stages of the cycle (Fig. 18).

In old hens, base-line plasma progesterone concentrations were significantly lower 4-9h after an ovulation than 30-33h after an ovulation ($P < .01$; unpaired t-test). The mean change in plasma progesterone concentrations was greater ($P < .05$, unpaired t-test) when the largest ovarian follicle was immature (4-9h after an ovulation) than when it was mature (30-33h after an ovulation), but the maximum change in plasma progesterone concentration, and the area under the curve did not differ with time after an ovulation. There was no significant difference in egg production between the two groups of old hens injected at different stages of the cycle (Fig. 19).

Although old birds produced significantly fewer eggs (65.0% v 78.0%; $P < .01$), there were no differences in the response variables (referred to above) between all old hens and all young hens. Comparisons between old and young birds injected 4-9h after an ovulation, and between old and young hens injected 30-33h after an ovulation likewise showed no significant differences in the response variables.

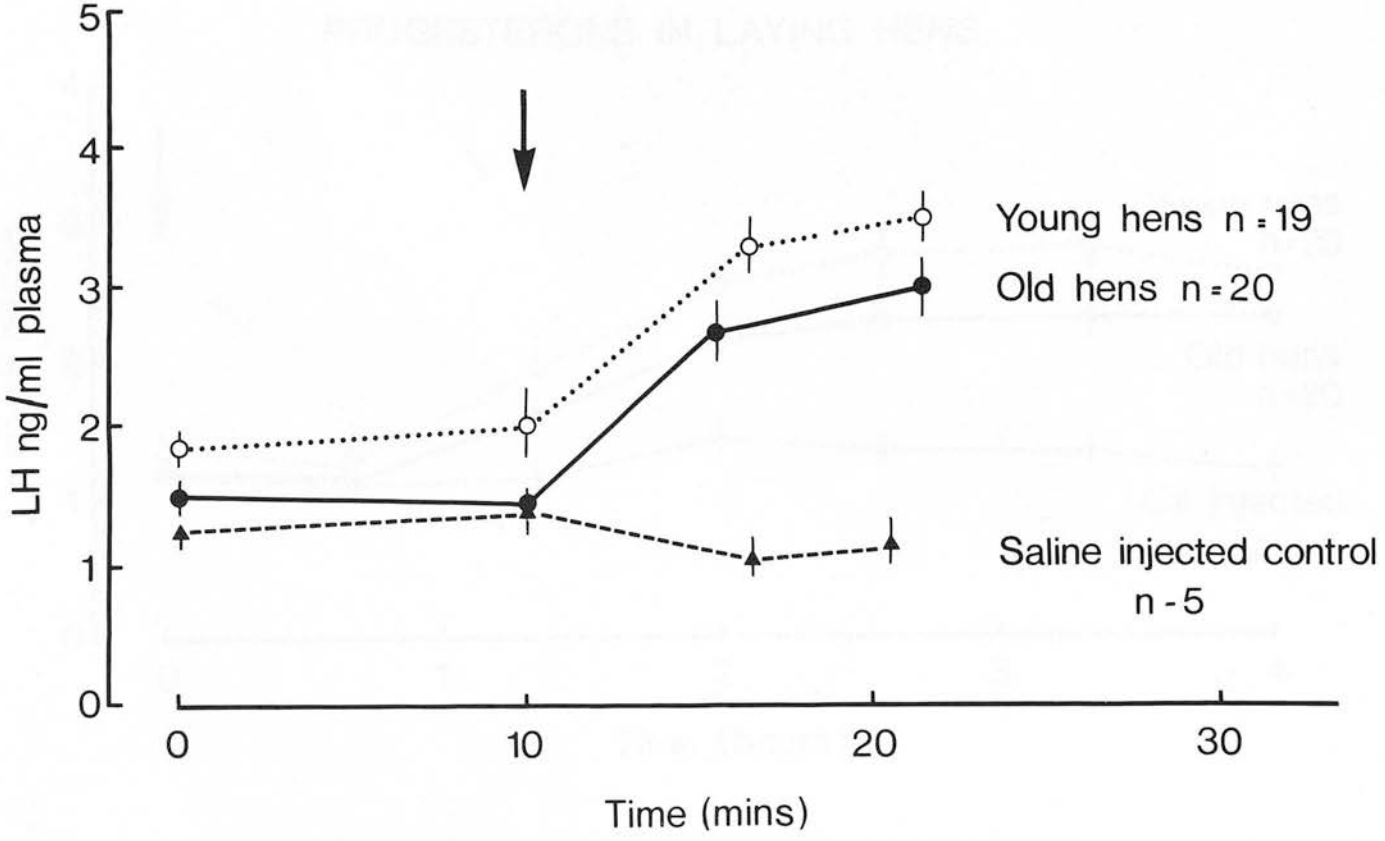
4.10.4
Base-line concentrations of LH

Higher base-line plasma LH concentrations were observed in young hens than in old hens in the experiment when progesterone was injected to test the sensitivity of the positive feedback system,

and also in the experiment when pituitary sensitivity was tested by LH-RH injection. In both cases, this difference was statistically significant ($P < .05$; unpaired t-tests).

Figure No. 15

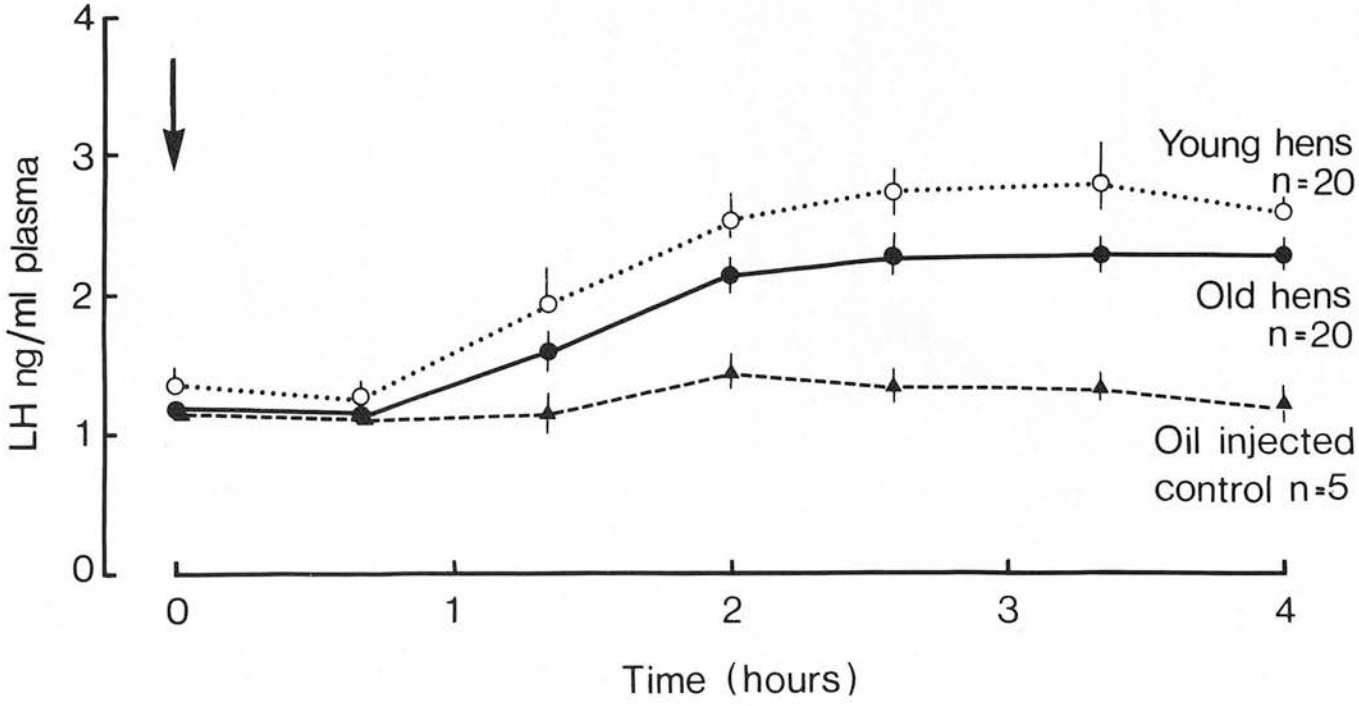
CHANGES IN PLASMA LH FOLLOWING AN INJECTION OF SYNTHETIC LHRH IN LAYING HENS.



Arrow indicates time of injection

Figure No. 16

CHANGES IN PLASMA LH FOLLOWING AN INJECTION OF PROGESTERONE IN LAYING HENS.



Arrow indicates time of injection

Figure 17

Dose-response relationships of progesterone plasma levels following an ovine LH injection given intravenously in laying hens.

Top three figures - injections given 26-30h after an ovulation
Next three figures - injections given 0-6h after an ovulation
Bottom figure - controls (any stage of ovulatory cycle)

PLASMA PROGESTERONE LEVELS FOLLOWING LH INJECTION (mean SEM)

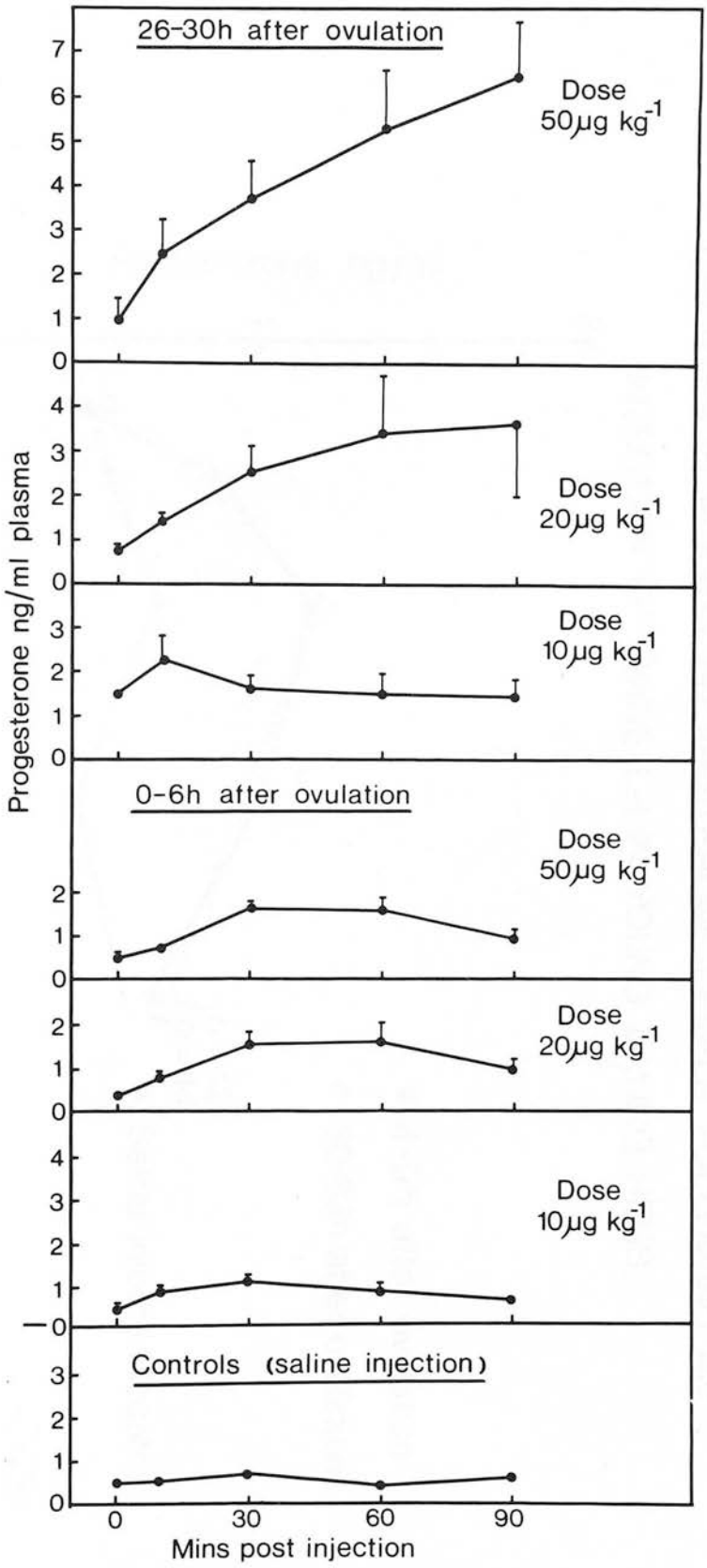


Figure No. 18

CHANGES IN PLASMA PROGESTERONE LEVELS FOLLOWING AN INJECTION OF OVINE LH IN YOUNG LAYING HENS.

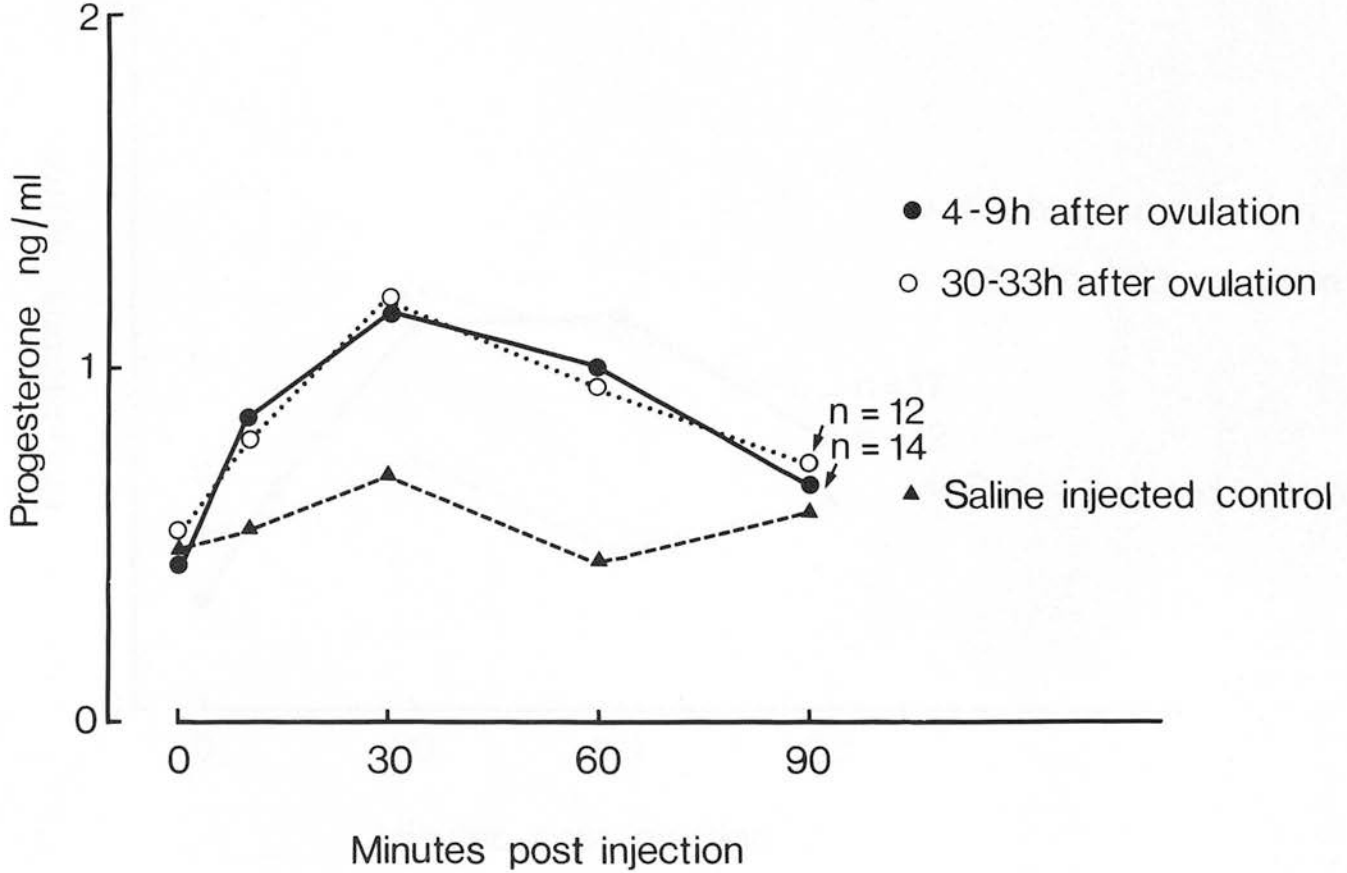
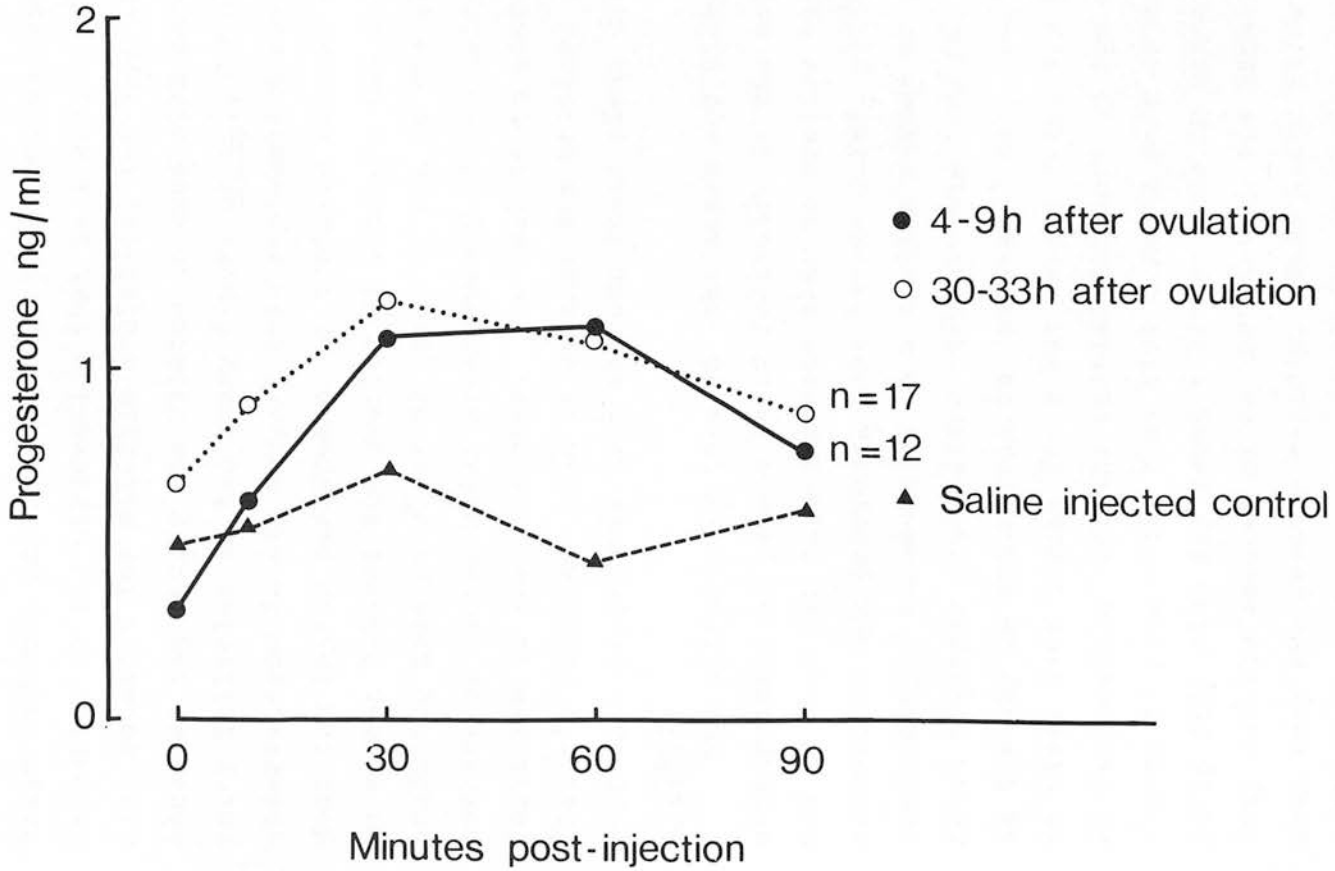


Figure No. 19

CHANGES IN PLASMA PROGESTERONE LEVELS FOLLOWING AN INJECTION OF OVINE LH IN OLD LAYING HENS.



Discussion

Jaap & Clancy (1968) found that the ovaries of broiler type hens contained more yellow yolky follicles than did those of commercial egg producing birds and that broilers laid more abnormal (i.e. membraneous, soft-shelled or double yolked) eggs than did commercial egg producers. This suggests that broilers lay less well because their ovaries ovulate more yolks than their oviducts can process into hard-shelled eggs. This was supported by the observation that in a strain of White Leghorn hens selected for multiple ovulation, the high incidence of abnormal egg laying was related to excessive numbers of yellow yolky follicles in the ovary (Sharp *et al.*, 1976). The above observations do not support this explanation for the relatively poor egg laying performance of broilers because no differences were seen between the ovaries of broilers and commercial egg producing hens at 26 or 82 weeks of age in the sizes, weights, numbers or hierarchical arrangements of the yellow yolky follicles in their ovaries. The failure to confirm Jaap & Clancy's findings could be because the broilers in this study were on a restricted diet whereas their birds were fed *ad libitum*.

The differences between the sizes and hierarchical arrangements of yellow yolky follicles in the ovaries of 26 and 82 week old hens indicate that as broiler breeder or commercial egg producing hens become older, yellow yolk increasingly accumulates in a smaller number of follicles which reach a larger size before they ovulate (Fig 14). This change in the way in which yolk is 'packaged' into follicles results in older hens laying fewer but larger eggs, and it may be partly responsible for the initial fall-off in the rate of lay in flocks of hens after they have reached peak production. Yellow yolky follicles may reach a larger size in older hens before they ovulate because of an increase in the duration of the phase when they are growing rapidly. This would allow more time for yolk to accumulate. Alternatively or additionally there may be an increase in the rate at which yolk is transported into the follicles of older hens. Since there was no difference between

the number of days taken for dye administered orally to disappear from the yolks of successive eggs laid by 26, 52, and 113 week old hens it is unlikely that the time taken for a follicle to reach an ovulable condition increased with the age of the bird. This is not in agreement with the findings of Lacassagne (1960) who used a more sophisticated version of the dye administration technique to compare the duration of the rapid growth phase of follicles in the same hens at two ages. He found that between 44 and 78 weeks of age the time taken for a follicle to reach an ovulable condition increased by about 22h. Warren & Conrad (1939) also used the dye administration technique to compare the rate of growth of the yellow yolky follicles in pullets when they first started to lay and in the same birds after they had been in lay for eight weeks. During this period the size of the yolks in the eggs increased by 29%. Warren & Conrad were unable to find any evidence that this increase was due to an accelerated rate of growth of yellow yolky follicles and concluded that the follicles were taking a longer time to grow to an ovulable size. The different methods of analysis and ages of birds used by Lacassagne and by Warren & Conrad make it difficult to compare their findings with the present experiment. Our observations support the data collected by Gilbert (1971b) which show that as laying hens become older the rate of transport of yellow yolk into rapidly growing ovarian follicles increases.

The initial fall-off in egg production with age may be primarily due to a reduction in the rate at which follicles enter their final growth phase. This is suggested by the observation that the ovaries of 82 week old broiler or commercial egg producing hens contained fewer yellow yolky follicles than the ovaries of 26 week old birds (Table17).

After feeding hens with dye, there were more eggs collected with dyed yolks from 26 and 52 week old hens than there were developing yellow yolky follicles in their ovaries (Table 18). This is consistent with the view that follicles enter their rapid growth phase when they are less than 9mm in diameter (Gilbert, 1971a).

The poor rate of lay in the 82 week old broiler breeder

or commercial egg producing hens laying at a rate of less than 50% was not due to a slowing down in the rate of recruitment of follicles into the rapid growth phase because the ovaries of these birds contained as many yellow yolky follicles as the ovaries of good layers of the same age. Neither was it due to gaps in the yellow-yolky follicular hierarchies. The most likely explanation is that many yellow yolky follicles failed to give rise to hard-shelled eggs. This is supported by the study on the deposition of dye in 113 week old hens (Table 19). These birds all had complete yellow yolky follicular hierarchies but were laying at a low rate. The discontinuities in the pattern of decreasing dye ring diameters in successive eggs produced by these birds show that failure to lay regularly was not due to a delay in the ovulation of mature ovarian follicles (Table 19). It can only be accounted for by the failure of some follicles to give rise to hard-shelled eggs. The fate of such follicles is a matter for conjecture but they may have become atretic or have ovulated yolks which failed to enter the oviduct. They may also have given rise to membraneous or soft-shelled eggs which fell through the cage bottom and were never recorded. Post-mortem examination of poor layers produced evidence for follicular atresia and internal ovulation but no quantitative observations were made. These observations support the suggestion that the poor rate of lay in many birds at the end of their first laying year is due to an increased incidence of internal laying (Wood-Gush & Gilbert, 1970). These authors arrived at this conclusion by showing that old layers often show nesting behaviour (which is associated with ovulation) without laying an egg.

In conclusion, the studies on ovarian structure and function suggest that as laying hens become older, the initial fall-off in egg production and the increase in egg size is a reflection of the way in which yellow yolk increasingly accumulates in a smaller number of follicles which grow to a larger size before they ovulate. A sharp fall-off in the rate of lay of individual hens towards the end of their first laying year is due to many yellow yolky follicles failing to ovulate or to produce yolks which reach the outside world in hard-shelled eggs.

In broiler breeders, this latter process starts to occur earlier in their laying year than in the commercial egg producing hens.

A decrease in the sensitivity of the LH positive feedback mechanism in ageing hens may be due to a change in the concentrations of oestrogen and progesterone in the blood, since in the ovariectomized hen the mechanism becomes functional only after 'priming' with closely defined doses of oestrogen and progesterone (Wilson & Sharp, 1976b). Any increase or decrease in the priming doses of these steroids, and in particular of progesterone, leads to a decrease in LH positive feedback response to progesterone. Since base-line concentrations of progesterone in the present study varied much more in old than in young hens (Figs 18 &19) this may cause a reduction in the sensitivity of the positive feedback mechanism.

The increase in the concentration of plasma progesterone after an injection of 20µg ovine LH was similar to the increase observed by Shahabi et al. (1975b) in response to an injection of 25 µg ovine LH 12h after oviposition. Etches & Cunningham (1976) investigated the capacity of the ovary to release progesterone in response to increased plasma LH caused by a subcutaneous injection of 25 µg LH-RH. Following an injection of LH-RH 6.5h after ovulation , there was a minor increase in the plasma progesterone concentration of approximately 0.3 ng/ml. Following an injection of 25µg LH-RH 27h after ovulation these authors observed a much larger and prolonged increase in the concentration of plasma progesterone which was associated with a steep increase in the plasma LH level. It thus seemed that when LH-RH was injected 27h but not 6.5h after ovulation it resulted in the secretion of sufficient progesterone to trigger the release of LH by positive feedback. This implies that between 6h and 27h after ovulation there is an increase in the capacity of the follicle next due to ovulate to secrete more progesterone in response to the same gonadotrophic stimulus. In the present study the concentration of plasma progesterone rose by about 0.7 ng/ml following an injection of LH 4-9h or 30-33 h after an ovulation. Such a change in the level of plasma progesterone would not consistently stimulate LH release by positive feedback (see p. 58). The

failure of injections of ovine LH to stimulate the secretion of more progesterone from a mature follicle than from an immature follicle suggests than a gonadotrophic stimulus in addition to LH is needed to stimulate maximum progesterone release from the maturing follicle. The possibility that ovine LH is sufficiently different from avian LH that it can never maximally stimulate the steroidogenic components of the ovary cannot be excluded either.

Alternatively, the dissimilar patterns of secretion of progesterone produced by subcutaneous LH-RH injection and intravenous administration of ovine LH may be explained by the different lengths of the period of stimulation of the ovary achieved by each method of hormone administration. In the above experiment, plasmas from 25 experimental hens were assayed for ovine LH, and peak values (mean = 212.4 ng/ml plasma) were attained 10 min post injection. At 90 min after injection plasma levels of ovine LH had fallen to 6% of peak levels. The levels of LH-RH in the blood following subcutaneous injection of the decapeptide remain a matter for conjecture, but it is generally accepted that this method of administration results in a more prolonged release of injected material into the bloodstream.

The observation that, in old hens, the maximum change in the concentration of plasma progesterone after an LH injection was greater at 4-9 h than at 30-33 h after an ovulation may be connected with the lower base-line concentration of plasma progesterone during the former period (Fig 19). In young hens the base-line concentration of plasma progesterone before injection of LH was the same 4-9 h and 30-33 h after ovulation (Fig 18).

The decrease in the base-line concentration of plasma LH in old laying hens (Figs 15 & 16) was not due to a decline in the responsiveness of the pituitary gland to LH-RH. It may thus reflect a decrease in the activity of the neural mechanisms controlling the basal secretion of LH-RH. A reduction in the rate of lay after hens have been laying for 2 or 3 months is due to a decrease in the rate of recruitment of follicles into the yellow yolky follicular hierarchy. (p 89). This reduced rate of recruitment could be partly caused by the decrease in base-

line levels of plasma LH.

In conclusion, the study of the hypothalamo-pituitary ovarian axis suggests that in the hen, as in the rat but not in women (Jones, 1975; Ascheim, 1976; Meites, Huang & Riegle, 1976), the decrease in reproductive activity with age is partly due to a functional change in the central nervous system and that the ovary remains potentially fully functional.

4.11. GENERAL DISCUSSION

4.11.1. Specificity of the LH Radioimmunoassay

The specificity of the assay described by Follett *et al.* depends on the use of a highly purified LH preparation as the ^{125}I labelled ligand. Ethanol-ammonium acetate extraction precipitated prolactin and growth hormone from the acetone dried chicken pituitary powder. The soluble glycoprotein fraction, follicle stimulating hormone (FSH), LH, Thyroid Stimulating Hormone (TSH), was chromatographed on CM cellulose to remove FSH. The fraction adsorbed on CM cellulose was subjected to further ion-exchange chromatography to separate LH from TSH (Stockell-Hartree & Cunningham, 1969). Follett *et al.* (1972) found that LH prepared in this way (IRC 2 fraction) was not devoid of TSH activity, and modified the final purification step to produce a fraction, AE 1, used for iodination. AE1 posessed a greater gonadotrophic:thyrotrophic ratio (assessed by bioassay methods) than IRC 2. Nonetheless, it was shown that antiserum raised to IRC 2 blocked the effect of TSH *in vivo*. Also, Follett *et al.* (1972) found that both thyroxine and thiourea lowered apparent LH levels in male quail, while in female quail thiourea depressed LH plasma levels and thyroxine increased plasma LH levels. It may be argued that these results do not constitute evidence for a consistent effect of manipulation of the pituitary-thyroid system on plasma LH levels. However, the possibility that the LH radioimmunoassay used in the work presented here partially measures TSH cannot be ruled out. Observations made on three female chickens radiothyroidectomized with ^{131}I showed that plasma LH levels increased post-thyroidectomy, and in one case the increase was tenfold (unpublished observations). The small number of animals used did not permit rigourous statistical comparisons and for this reason the experiment was not reported in the results section of this thesis. Radioimmunoassay is one of the most powerful tools available to researchers in endocrinology, but these results show that some caution must be exercised in the interpretation of results obtained using the assay described by

Follett et al. (1972).

4.11.2. Progesterone Radioimmunoassay

This procedure was largely satisfactory but two related problems were encountered in routine checks performed to validate the assay when it was first set up in the P.R.C. laboratories. These were : A low rate of extraction of hormone ; non-parallelism of plasma extracts and the standard curve. An investigation of the former problem revealed that extraction of progesterone into light petroleum was quantitative, but resolubilization in the aqueous RIA diluent was not. Two possible factors causing this problem were the extreme insolubility of progesterone in polar solvents and a tendency for progesterone to be adsorbed on glass. These factors have been referred to by Orczyk et al. (1974) who have described a protocol for the extraction of progesterone from plasma which results in a near quantitative recovery in the aqueous assay diluent. The second problem, that of non-parallelism of plasma extracts with the standard curve, persisted throughout the whole series of assays used in these experiments, although the assay protocol used minimized this to an acceptable extent. In routine assays, non-parallelism was only noticeable above 320 pg/tube and the effect was minimized by the adoption of a range of internal standards in each assay. These were extracted in the same way as plasma samples and the data so obtained was used to apply correction for recovery of progesterone from plasma and to correct for the limited non-parallelism that was encountered. The problem of non-parallelism has been discussed by Rolleri & Malvano (1974). The extent of non-parallelism encountered by these authors in progesterone radioimmunoassays was similar to the assays used in the work presented here. While the insolubility of progesterone in aqueous media, and adsorbtion to glass could account for this, Rolleri & Malvano (1974) have drawn attention to the possible interference of sovent residues originating from the extraction step. These authors have also shown that in the case of testosterone, where three different antisera were examined, the avidity of

the antiserum is an extremely important factor. These observations underline the crucial importance of validating any steroid radioimmunoassay when it is used in a different laboratory. In addition, much effort has been directed towards the development of highly specific antisera recently. While this is highly desirable, the performance of the antiserum under assay conditions should be examined and the choice between equally specific antisera for use in RIA work could be made on the results of parallelism tests of plasma extracts and the standard curve.

4.11.3. The Ovary and Rates of Egg Laying

In the foregoing discussions, the 'ovulability' of a follicle was suggested to depend on its ability to secrete, and sustain secretion of, progesterone in response to a diurnal increase in the plasma levels of LH and/or other stimuli. When this progesterone secretion is sufficient to trigger the pre-ovulatory release of LH by positive feedback, the follicle can be said to have attained the state of minimum ovulability. As hens age, they tend to lay shorter sequences of eggs which indicates that the interval between successive follicles attaining minimum ovulability tends to lengthen. Although follicles attain a greater size and weight before ovulating in older hens, this change in the follicular growth process does not reflect the change in the rate of attainment of minimum ovulability. First, the duration of the rapid growth phase remains unchanged and secondly, hens ovulating larger follicles could lay at very high rates (pp90 & 81). Further evidence can be found in the data of Sharp et al. (1976). Follicles were ovulated at a much lower weight in multiple ovulating hens than in a normal, single ovulating strain of the same age. It therefore seems that the factors controlling growth of the follicle and yolk deposition are not the same as those controlling the development of ovulability.

Just prior to ovulation, progesterone content rises in the largest follicle, testosterone content rises in the three largest follicles with the largest rise in the second largest

follicle, and oestrogen content rises in the third largest follicle (Shahabi et al., 1975a). While these results are indicative of a maturation process in the steroid synthesizing capacity of the hen's ovarian follicle, Shahabi et al. (1975a) did not measure the steroid hormone content of the granulosa cell layer since this would have been lost when they removed the yolk from the follicle (Gilbert et al., 1977). The granulosa cells are probably important sites of steroid synthesis in the hen's pre-ovulatory follicle but this needs to be confirmed. It is generally accepted that luteinized granulosa cells of the mammalian follicle secrete progesterone (Channing, 1970 ; McNatty et al., 1974 ; Moor et al., 1975). In the sheep, granulosa cells start to secrete large amounts of progesterone 12 to 18 h before ovulation. Also, in the reptile, granulosa cells may be the site of progesterone production (Crews & Licht, 1975).

The changes in the steroid content of the theca of the hen's pre-ovulatory follicle during the three days preceding ovulation (Shahabi et al., 1975a) follow a similar time course and pattern to the series of changes in steroid secretion observed in explanted sheep follicles in vitro. The highest levels of oestrogen were produced by follicles explanted on day 14 of the oestrous cycle. Oestrogen production declined rapidly late in day 15 in parallel with an increased production of testosterone but on day 16 (oestrous), oestrogen and testosterone production declined sharply and large amounts of progesterone then became the principal secretory products (Seamark et al., 1974). These authors have pointed out that the change from the secretion of oestrogen to the secretion of progesterone as a follicle matures is accompanied by a decrease in synthesizing capacity in the theca and an increase in steroidogenic activity of the granulosa. It is possible that the increased progesterone content of the theca of the largest follicle observed just before ovulation in the hen is the result of secretion by the granulosa cells, because progesterone produced by the granulosa cells would have to be transported across the thecal layer of the follicle wall to reach the vascular system. The question arises whether follicles in older hens might switch to increased

progesterone production at a later stage in their development than follicles in young hens, and a knowledge of the factors controlling the change in steroid synthesis may be relevant to a deeper insight into the meaning of ovulability of a follicle.

The decrease in the sensitivity of the progesterone induced positive feedback mechanism governing LH secretion in ageing hens may also lead to a shortening of egg sequences. This mechanism appears to be dose-responsive (Wilson & Sharp, 1975b) so the possibility arises that the minimum amount of progesterone required to stimulate the LH secretory mechanism is greater in older hens. Thus, the follicles of older hens may mature at a relatively later stage in the ovulatory cycle because they need to secrete greater amounts of progesterone in order to trigger a pre-ovulatory surge of LH than do follicles in young hens. Basal plasma progesterone levels were higher in old hens when the ovary contained a mature follicle compared to young hens, but this does not necessarily argue against a protracted maturation time. These higher basal levels were observed before the onset of the pre-ovulatory surge when the progesterone content of the follicle increases so dramatically. An interpretation is that these higher basal plasma progesterone levels may result from a greater number of steroid-secreting cells in the larger follicles of older hens. The 'ovulability' of the follicle may be related to the capacity of the follicle to secrete large amounts of progesterone sufficient to trigger positive feedback, and this occurs at a later stage in the ovulatory cycle than the time at which the observations on basal plasma progesterone levels were made.

The experiments presented above showed that abnormal laying patterns were partly responsible for declining egg production in the first laying year, and were probably due to the laying of membraneous or soft-shelled eggs, internal ovulation or follicular atresia. The laying of membraneous and shell-less eggs may be attributed to oviduct malfunction. It is uncertain, however, to what extent abnormal laying patterns are caused by environmental factors such as nutrition, lighting and temperature on the one hand, and to genetic factors on the other. A marked drop in production in month 4 of

lay was associated with the cessation of normal, coupled sequences in Babcock and Ranger hens. Ross I hens showed a drop in production too, which suggested that a common environmental factor was responsible. However, not all Ross I hens stopped laying coupled sequences. This fact may be related to the different genetic constitution of these hens, as may the observation that abnormal laying patterns were, on average, more prevalent in broilers than egg-type strains during the first laying year. In support of the idea that abnormal egg laying patterns may be genetically controlled, Guillaume (1976) noted that dwarf broiler strains were not less productive than normal broiler hens, even though they lay shorter sequences (Prod'homme & Mérat, 1969). They are just as productive as full-size broilers because they lay regular, coupled sequences and show no evidence of abnormal egg laying. Nevertheless, the experience of the poultry industry suggests that broiler egg production can be affected by manipulating the environment. The company from whom the broilers were purchased recommends feed restriction with the expressed aim of limiting body weight, delaying puberty, and improving egg production. Feed restriction was practised on the broiler hens used in this study, but the absence of a control group makes it difficult to assess if this measure reduced the incidence of abnormal laying patterns. Jaap & Muir (1968) studied laying patterns in broiler hens and noted that 37% of the membraneous eggs were laid on the day preceding a pause day. However, a membraneous egg was often followed by a second egg laid the same day, and it is possible that a membraneous egg may be expelled prematurely due to the pres ence of a second ovum in the uterus. These authors' observation that two eggs (sometimes both hard-shelled) can be laid on the same day is interesting. Possibly two ovulations occur in the same open period, or the timing of ovulations may be highly irregular because the mechanism controlling the occurrence of the open period is defective. It is unlikely that double ovulations were an important cause of abnormal laying patterns in the broilers studied here, as the examination of their ovaries showed them to contain a graded hierarchy of single follicles whereas the ovaries of hens selected for

double ovulation contained hierarchies of pairs of equally sized follicles (Sharp et al., 1976). The case for oviduct malfunction being the cause of abnormal laying is strengthened by evidence of internal ovulation in old broiler hens examined post mortem (p 83).

Follicular atresia was observed in many hens at post-mortem, but never in hens aged less than 52 weeks. Also, atresia was commonly observed in the mass of small white follicles and only occasionally were large, yellow atretic follicles seen in the hierarchy of large follicles. In contrast, large atretic follicles were seen in grouse killed at the end of the breeding season but this may be associated with the withdrawal of gonadotrophic support. In some respects, it is the non-occurrence of visible atretic follicles in young hens that is the surprising observation. In mice (Peters, 1976) and sheep (Hay & Moor, 1975) many more growing follicles become atretic than mature and ovulate. Thus, the normal fate of ovarian follicles in mammals is to become atretic rather than to develop and ovulate. Is this true of the hen's ovary? It is known that only a small number of the total population of oocytes develop to the point of ovulation (Fauré-Fremiet & Kaufmann, 1928 ; Zander et al., 1942). Brambell (1926) has reported atresia in the largest follicles of 42 and 77 day old chicks, but no quantitative data are available indicating the relative occurrence of atresia and healthy development of oocytes. It may be that in the case of the young (26 w.o.) hens atresia was present but in the microscopic follicles not visible to the naked eye. It has been shown in the mouse that PMSG and FSH prevent atresia in vivo (Peters, 1976). A similar effect has been demonstrated in the sheep (Hay & Moor, 1975) and the effect appears to depend on the time of exposure of the follicle to gonadotrophins in vivo. It has also been shown that FSH, but not LH, maintains the viability of human granulosa cells in culture (McNatty et al., 1975). Thus, although basal LH levels in plasma of laying hens were found to decrease with age, this may have no effect on the incidence of follicular atresia. However, if plasma FSH levels are depressed in ageing hens, this could result in increased follicular

atresia. The administration of partially purified avian FSH did not prevent, and only delayed ovarian regression in acutely hypophysectomized hens ; neither did FSH induce development in the long-term hypophysectomized hen (Mitchell, 1970). On the other hand, ovarian development was induced in hypophysectomized hens by treatment with crude chicken pituitary extract (Mitchell, 1967a). These data suggest that development of follicles to an ovulable condition depends on both FSH and LH, and one wonders if the levels of these two hormones in the circulation might affect the rate of follicular atresia as well.

De Reviers & Terqui (1976) have studied the development of the ovarian follicles in three strains of rat shown previously to have different levels of sensitivity to FSH in the Steelman-pohley ovarian augmentation bioassay. Commenting on these results, and studies on pituitary and circulating levels of FSH and LH carried out in parallel, these authors have postulated that the observed differences in the proportion of primordial follicles (% of the total population) which start development postnatally may be attributed to the different ratios of FSH to LH, and/or the absolute levels of each hormone observed in each strain, although differences in FSH receptors at the ovarian level are not excluded. However, the total number of developing follicles also depended on the absolute number of primordial follicles present in the ovary before development commenced. Thus, a strain with a low sensitivity to FSH, and a large number of primordial follicles was observed to have a similar number of follicles undergoing development as a strain with greater sensitivity to FSH and a smaller number of primordial follicles. The three strains of hen studied in this thesis were shown to have differing levels and patterns of LH secretion during sexual development and while the absence of FSH data makes comparison with the foregoing experiments on the different strains of rat difficult, one may speculate on the possible relationship between hormone levels and ovarian development in the pre-pubertal period, and subsequent reproductive performance. Romanoff & Romanoff (1949) have commented that the number of oocytes visible to the naked eye in hens in their first year of laying is far greater than the number of eggs laid in the

lifetime of a hen. However, these authors have also demonstrated that more oocytes are visible in lightweight breeds than in heavyweight breeds. It was postulated earlier that a slowing of the rate of recruitment of follicles into the rapidly growing hierarchy of yellow, yolky follicles could cause a decline in the rate of laying. It was also suggested that age-related changes in the follicular hierarchy may be evident at an earlier stage in the life of broiler-type hens. The question which may be posed is 'Does the rate of recruitment depend on the size of the pool of follicles from which follicles are selected?' It may be that the slow growth period of oocytes which starts one week before the chick hatches and lasts for months or years (Romanoff, 1960) may have a bearing on subsequent reproductive performance. The factors which might be important include the number of oocytes present in each strain before this development commences, their sensitivity to, and levels of, gonadotrophin during subsequent development as well as the proportion of follicles becoming atretic and the proportion developing to an ovulable condition.

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PUBLICATIONS

Material included in this thesis has been submitted or accepted for publication as the following papers:

Williams J.B. & Sharp P.J.
Comparison of plasma progesterone and plasma luteinizing hormone in growing hens from eight weeks of age to sexual maturity.
J.Endocr.

Williams J.B. & Sharp P.J.
Control of the pre-ovulatory luteinizing hormone surge in the chicken, Gallus domesticus : the role of progesterone and androgens.
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Williams J.B. & Sharp P.J.
Age-dependent changes in the hypothalamo-pituitary ovarian axis in the laying hen.
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Williams J.B. & Sharp P.J.
Ovarian morphology and rates of ovarian follicular development in laying broiler breeder and commercial egg-producing hens.
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